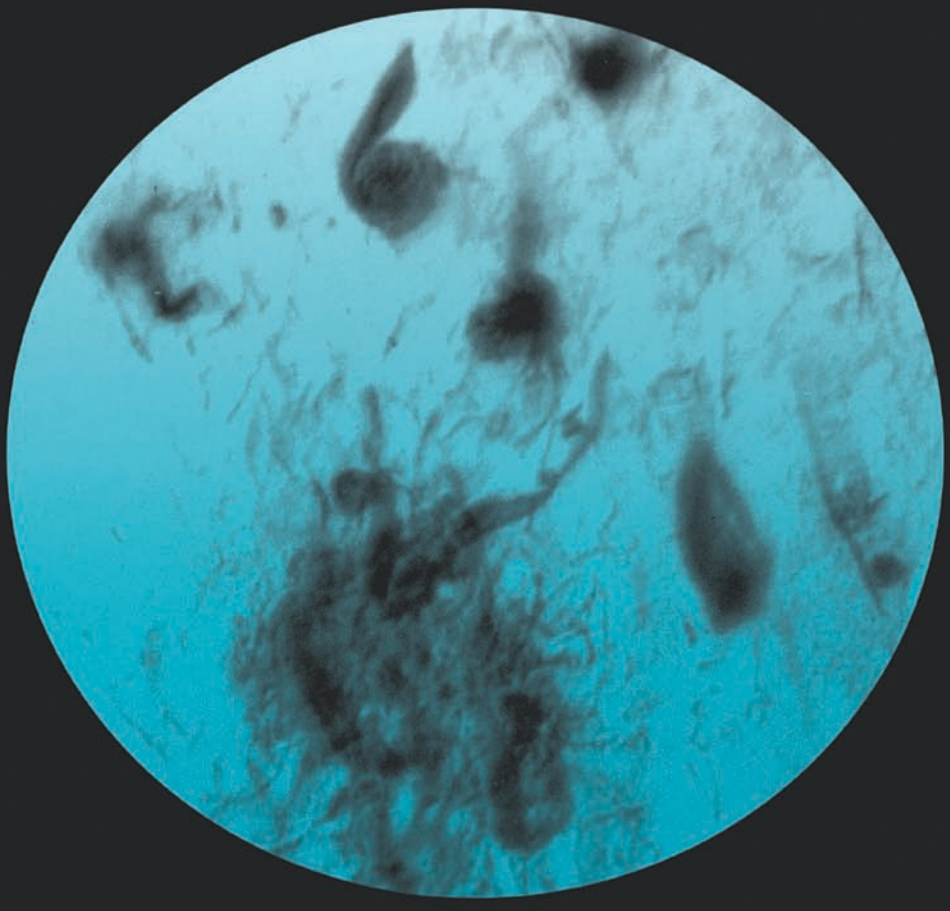
# Alzheimer Disease

## Current Research in Early Diagnosis

Edited by Robert E. Becker and Ezio Giacobini



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| USA | Publishing Office: | Taylor & Francis New York Inc.<br>79 Madison Ave., New York, NY 10016-7892 |
| | Sales Office: | Taylor & Francis Inc.<br>1900 Frost Road, Bristol, PA 19007-1598 |
| UK | | Taylor & Francis Ltd.<br>4 John St., London WC1N 2ET |


**Alzheimer Disease: Current Research in Early Diagnosis**


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First published 1990
Printed in the United States of America

**Library of Congress Cataloging in Publication Data**

Alzheimer disease : current research in early diagnosis / edited by
Robert Becker and Ezio Giacobini.
p. cm.
Includes bibliographical references.
ISBN 0-8448-1659-0 (hc)
1. Alzheimer's disease--Diagnosis. I. Becker, Robert E.
II. Giacobini, Ezio.
[ DNLM: 1. Alzheimer's Disease--diagnosis. WM 220 A475 ]
RC523.A377 1990
618.97'6831'075--dc20
DNLM/DLC
for Library of Congress 90-11067
CIP

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# Preface

This book addresses the early clinical diagnosis of Alzheimer disease. It was written to evaluate the relevance of current research for the early diagnosis of Alzheimer disease. The process of creating this book was informative to the authors who now appreciate that improvements in diagnostic clinical criteria have already made earlier diagnosis more available. The process of review of current research also uncovered the present lack of clinical utility in research methodologies such as neuroimaging, drug challenges, electroencephalographic studies and brain biopsy.

It is hoped that the essays addressing each of these issues will serve two purposes for the reader, 1) to provide a useful perspective on the role of each technology in clinical practice, and 2) to provide to both the interested medical generalist and the specialized researcher an in-depth introduction to the technologies and research findings in each of the areas reviewed.

Robert E. Becker
Ezio Giacobini



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# Introduction

Robert E. Becker
*Department of Pyschiatry, School of Medicine*
*Southern Illinois University, Springfield, IL*

Alzheimer disease (AD) is becoming a major health care problem as increasing numbers of persons are diagnosed. In the near future a very significant proportion of the elderly population will be affected by AD. The estimated incidence rates increase sharply from 1% at 65 to 20% at 85. But these present estimates of increased age specific incidence rates may be incorrect, it has recently been reported in a community prevalence study that nearly half of the population over age 85 met diagnostic criteria for AD (Evans et al., 1989). This implies that as the population ages AD may become a very common family tragedy.

During the decade of the 80's, public awareness of the increasing prevalence of AD was accompanied by public concern for the development of improved diagnosis, treatment and management of patients and by public calls for increased support for families with an affected member. In the last decade, we have successfully exposed as a myth the belief that elderly are mentally impaired as a result of aging. Complaints of cognitive loss in the aged now are recognized as indicators of a disease process that requires exact diagnosis. This has changed medical practice, the physician who sees elderly patients must be prepared to pursue the differential diagnosis of the demented patient until all of the many treatable causes have been excluded.

The most common diagnosis reached in the demented elderly patient is AD. But it is reached partly as the result of the successful exclusion of treatable forms of dementia. Treatable forms of dementia account for up to 50% of all patients who present at the physician's office with complaints of impaired memory. This process of clinical diagnosis is described and the appropriateness and utility of various laboratory procedures are discussed by Kawas and Elble in their chapters that address early clinical diagnosis.

From these chapters the reader with a primary clinical concern can move to more indepth discussion of specific technologies in the other chapters. A similar approach to the book could be taken by the research scientist interested in understanding the relation of his

field to present diagnostic practice or the potential contribution of other fields to the problem of early diagnosis.

We will view this book as successful if it provides practical clinical guidance to physicians responsible for diagnosis of demented patients. In addition to providing practical clinical guidance to the practitioner, this book can be used by the research specialist to put his and other fields into a clinical perspective. But there are two other issues that we address. What is the stage of progression at which diagnosis can currently be reached and what potentials exist in modern technologies to achieve even earlier diagnosis?

## IS EARLY DIAGNOSIS EARLY ENOUGH?

As many authors in this volume document, it is clear that with improved clinical and neuropathological criteria for the diagnosis of AD the percentage agreement between clinical diagnosis and post-mortem diagnosis has improved in the last decade. Early studies reported less than 50% agreement and more recent studies range 80-100% agreement (Morris et al., 1988). The very high agreement in recent studies may be an artifact of carefully selected research populations being used and of the requirement for diagnosis at post-mortem examination that a clinical dementia be present (Khachaturian, 1985). This improved agreement between clinical diagnosis and post-mortem diagnosis is challenged by a recent report of only 68% agreement of NINCDS (McKhann et al., 1984) and Khachaturian (1985) diagnoses in a group of early onset patients diagnosed at relatively advanced stages of their illnesses (Risse et al., 1990). Most evidence supports the position that more effective diagnosis is being carried out and it is not unreasonable to propose that AD is being correctly diagnosed more frequently and as a result earlier in the course of the disease.

But is this early enough? There was both professional disinterest in this diagnosis and a reluctance to be invasive based upon the observation that there are no therapies available and an exact diagnosis has no useful implications for the patient. But this may change. As we have documented in the earlier volume in this series, Current Research in Alzheimer Disease (Giacobini and Becker, 1988), there is data to suggest that palliative therapies may become available within the next few years. Our group has been working with five different cholinesterase inhibitors (ChEI), physostigmine, metrifonate, heptylphysostigmine, THA and the huperzines, to evaluate the ChEI class of compounds for possible therapeutic efficacy and to understand the contribution of the cholinergic deficit to the cognitive pathology of Alzheimer disease. We have reported possible efficacy for improving cognition in AD patients with two inhibitors, physostigmine and metrifonate (Elble et al., 1988 and Becker et al., 1990) and have studies of efficacy underway for heptylphysostigmine. One other issue of interest is the effect of long term steady state cholinesterase inhibition on the progressive deterioration that is characteristic of AD. Unfortunately, our conclusion has been that this hypothesis cannot at present be tested because of methodological limitations. The sum of the variability in patient neuropsychological test scores, variability among patients in the rate and degrees of deterioration, and the expected mean group differences predicts the need for inordinately large group sizes and long durations of treatment to achieve adequate power to test the hypothesis using a double blind placebo controlled

trial. Therefore, we foresee no research data that can be developed in the near future to indicate whether a cholinomimetic treatment is useful to modify the course of the disease. Long term use of a drug shown to have palliative efficacy in AD will be of immediate interest to AD patients or those suspected of having AD since it could be hoped that a deteriorating course might be arrested as early as possible before functions have been irreversibly lost. Therefore, we fear that the appearance of the first approved drug for AD will suddenly widen the demand for earlier diagnosis of AD with the hope of patients and family members that "that things will not get worse" if early treatment is initiated. If this were to occur how will medicine respond? Not only may maintenance efficacy data for a drug not be available but earlier diagnosis may not be possible.

## CAN EARLIER DIAGNOSIS BE REACHED?

We suggest a reading of this volume leads to the conclusion that properly reached clinical diagnosis (i.e., discriminating use of clinical skills and laboratory examinations) can lead to exact diagnosis but cannot at present reliably produce earlier diagnoses. Present NINCDS criteria (McKhann et al., 1984) require the presence of cognitive and functional deficits that are socially and vocationally disabling. Without a "litmus test" -- and the chapters in this volume on neuropsychology, imaging, possible chemical or immunological markers document that there is none at present -- there will be disability before diagnostic criteria can be met. The appearance of definite and disabling cognitive pathology probably means the disease process must be relatively well established and, therefore, the possibility of preclinical diagnosis is excluded.

An early clinical diagnosis or late preclinical diagnosis may still be too late for effective therapy. The neuropathological lesions may have progressed without the appearance of behavioral change leaving too little intact tissue to benefit from a pharmacological intervention. In Parkinson disease, it has been estimated that an 80% loss of dopaminergic cells occurs in the substantia nigra before the appearance of clinically detectable behavioral changes. If AD clinically appears only after an anatomic deficit of comparable severity has become established then some means of diagnosis other than one dependent upon the appearance of early or even preclinical signs of dementia would be necessary.

## TECHNOLOGIES FOR AN EARLY DIAGNOSIS

Reading this volume suggests two further general problems that affect each of the technologies as they are applied to the early diagnosis of AD.

One problem is moving from the characterization of AD patients as a group to the diagnosis of individuals, i.e., assignment of an individual as an Alzheimer affected or non-affected person as distinguished from identifying group differences. At present, no method of measurement applied to AD can assign affected and non-affected persons to a bimodal non-overlapping distribution, or in other words, to one of two separate non-overlapping distributions. There are always "gray" cases, persons with values that fall within both the affected and non-affected groups, or in other words the two

groups overlap. Neurochemical determinations, drug challenges, MRI scans can indicate group differences but cannot at present reliably assign an individual as affected or non-affected.

Even though some tests have been reported to have very low percentages of misassignment, the frequency of AD in the population magnifies this error and prevents the test from being clinically useful. Take the following example: assume a test for AD will only falsely identify, as an AD patient, 1 out of 10 non-affected persons. And it will correctly diagnose 9 out of 10 persons with AD. At age 65, 1% of the population is affected, 99 out of 100 persons at age 65 are unaffected. Therefore, while we probably (only a 10% chance of omission) correctly identify the affected person, in the process we misdiagnose 10 other unaffected persons. By age 75, with a 10% prevalence of AD the situation improves, we diagnosis 9 or 10 affected persons out of every hundred we screen but we still misdiagnose 9 other unaffected persons (i.e., we have a 50% change of being right or wrong). One has the same probability of being correct or incorrect about the diagnosis of AD in demented patients by just flipping a coin since 50% of dementias are AD. The reader will see that no laboratory procedure presently available can overcome these consequences of limited sensitivity and specificity of tests which assign affected and non-affected populations to overlapping distributions.

## ALZHEIMER DISEASE OR ALZHEIMER DISORDER?

A second factor that is not widely considered, but in my view has potentially important implications, is that the affected person is mislabeled by being designated as affected by Alzheimer disease rather than Alzheimer disorder. In medicine, the category disease means that the etiology and pathogenetic mechanisms, by which clinical manifestations are produced, are known. When a patient exhibits a constellation of clinical signs and symptoms that are characteristic of a specific diagnosis, we properly say that the clinical syndrome supports the diagnosis. But the diagnosis that is supported may be a syndrome (e.g., jaundice), a disorder or two or more syndromes that occur together (e.g., schizophrenia), or a disease (e.g., pneumococcal pneumonia). It may be necessary to keep in mind that we do not know that AD is a single disease (i.e., has only one etiology and pathogenesis). It may be a syndrome that is the final common clinical pathway for a number of different pathological processes. The implications for further research are great. In studies that contrast affected and non-affected persons, the discovery of mechanisms of disease is dependent upon having a homogeneous group of affected persons to study. If the affected group that is to be studied shares the features of a clinical syndrome but the various individual members express the syndrome as the result of different pathological mechanisms, then the variance in pathological measures among group members will interfere with the detection of mean differences between the study and control groups. It may be that more efforts in Alzheimer "disease" research could be usefully turned to investigating if it is really Alzheimer disorder and to the identification of homogeneous subgroups of the disorder for study of disease mechanisms. If AD is a heterogenous group then successful early diagnosis research may await the successful characterization of homogeneous subgroups for which distinguishing features can be identified and then developed into reliable diagnostic criteria.

## CONCLUSION

At present, the clinical and pathological criteria used to diagnose AD are working criteria in the sense that further research will be needed to improve specificity and sensitivity. Because of the lack of treatment for AD, there may be no immediate benefit to patients from reaching an earlier diagnosis. This may change if any of the palliative therapies presently being studied are found to be effective. Earlier diagnosis could be of assistance to researchers as could more exact diagnosis because of the increased homogeneity of groups of affected persons available for study.

A wide range of technologies are being used to investigate Alzheimer disease. While many studies show significant group differences between Alzheimer disease patients and age matched controls, no one technique is sufficiently discriminating that it is at present useful as diagnostic marker for Alzheimer disease. Therefore, the diagnosis of Alzheimer disease will remain primarily a process of exclusion of treatable forms of dementia. In order for more specific criteria for Alzheimer disease to emerge, it may be necessary to determine if a single etiology or more than one pathological process is producing the clinical syndrome we presently call Alzheimer disease.

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# Part 1

# Early Clinical Diagnosis



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# Early Clinical Diagnosis:
## Status of NINCDS-ARDA Criteria

Claudia H. Kawas
*Department of Neurology, Johns Hopkins University*
*Baltimore, MD*

### Introduction

During the past decade there has been a significant improvement in the accuracy of the clinical diagnosis of Alzheimer's disease (AD). Whereas early series of diagnostic accuracy reported error rates of 30-40% (Marsden and Harrison, 1972; Rodron, et al., 1975; and Nott and Fleminger, 1975), more recent studies have verified the correct diagnosis over 90% of the time (Martin, et al., 1987; Morris, et al., 1988). This improvement has largely resulted from the development of specific criteria for AD which specify inclusion criteria and appropriate evaluation, rather than merely excluding so-called 'treatable' dementias and designating the remainder as AD.

The criteria currently recommended for use in theraputic trials were developed by the Work Group on the Diagnosis of Alzheimer's Disease established by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) (McKhann, et al., 1984). The intention was to provide uniform definitions for meaningful comparisons in theraputic trials regarding diagnosis and response to treatment. The criteria were also designed to be useful for comparative studies of AD patients in other types of investigations including case control studies, evaluation of new diagnostic laboratory tests, and clinicopathologic correlations. It was recognized that insufficient knowledge about the disease made the criteria tentative and subject to change after additional studies for validation had been performed.

### Criteria of the NINCDS-ADRDA Work Group

In 1984, the NINCDS-ADRDA Work Group published inclusion/exclusion criteria for the categories of DEFINITE, PROBABLE and POSSIBLE Alzheimer's Disease. These are outlined

Table 1. NINCDS-ADRDA Criteria for the clinical diagnosis of Alzheimer's Disease

I. The criteria for the clinical diagnosis of PROBABLE Alzheimer's disease include:

dementia established by clinical examination and documented by the Mini-Mental Test,(Folstein, et al., 1975) Blessed Dementia Scale,(Blessed, et al., 1968) or some similar examination, and confirmed by neuropsychological tests;

deficits in two or more areas of cognition;

progressive worsening of memory and other cognitive functions;

no disturbance of consciousness;

onset between ages 40 and 90, most often after age 65; and

absence of systemic disorders or other brain diseases that of themselves could account for the progressive deficits.

The diagnosis of PROBABLE Alzheimer's disease is supported by:

progressive deterioration of specific cognitive functions;

impaired activities of daily living;

family history of similar disorders; and

laboratory results of:
- normal lumbar puncture,
- normal pattern or nonspecific changes in EEG, and
- evidence of cerebral atrophy on CT with progression.

II. Clinical diagnosis of POSSIBLE Alzheimer's disease:

may be made on the basis of the dementia syndrome, in the absence of other neurologic, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in the onset, in the presentation, or in the clinical course;

may be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of the dementia.

III. Critieria for diagnosis of DEFINITE Alzheimer's disease are:

the clinical criteria for probable Alzheimer's disease and histopathologic evidence obtained from a biopsy or autopsy.

in Table 1. DEFINITE AD requires histological (autopsy or biopsy) confirmation and is therefore rarely useful in early diagnosis. The diagnosis of PROBABLE AD is made if there is a typical insidious onset of dementia with progression, and if there are no other diseases which could account for the deficits. Motor, sensory, or coordination deficits should not occur early in the disease. The diagnosis of POSSIBLE AD is utilized for cases where the presentation or course is somewhat aberrant. No prospective studies of unselected populations currently exist showing the relative percentages of each of these categories among AD patients. Theraputic trials generally require patients to have PROBABLE (or typical) AD.

## Comparison of NINCDS-ADRDA Criteria with DSMIIIR Criteria for Primary Degenerative Dementia

Prior to the development of the NINCDS-ADRDA criteria, the most commonly used definitions were those of the current Diagnostic and Statistical Manual of Mental Disorders (DSMIIIR) for Primary Degenerative Dementia. While compatible, there are several notable differences in the NINCDS-ADRDA criteria:

1) At least two areas of cognition (unspecified) are required for diagnosis but, unlike the DSMIIIR, memory does not necessarily have to be one of them.

2) Intellectual dysfunction must be demonstrable on formal mental status/neuropsychological testing. Major cognitive processes that are impaired in AD are specified and examples of tests are provided for assessing each process. See Table 2.

3) Specific evidence of deterioration in social or occupational functioning is not required.

4) Other DSMIIIR diagnoses, such as depression, do not have to be excluded to make a diagnosis of AD.

The advantages and disadvantages of these changes will be discussed later.

## Validity Studies: NINCDS-ADRDA Criteria

### Clinicopathological Studies

Several clinicopathological series (Joachim, et al., 1988; Morris, et al.,1988; Tierney, et al., 1988; and Wade, et al., 1987) have recently reported excellent validity for NINCDS-ADRDA criteria. Morris, et al., (1988) noted 100% accuracy in a clinicopathological series of 26 post-mortem examinations from a longitudinally studied research cohort. Seventeen of these individuals were diagnosed when only 'mildly' demented. Martin, et al., (1987) performed cortical biopsies on 11 patients with PROBABLE AD and also reported

Table 2. Recommended Neuropsychological Evaluation (NINCDS-ADRDA) (McKhann, et al., 1984)

The major cognitive processes that are impaired in Alzheimer's disease, with examples of the kinds of tests used to assess these functions, include:

orientation to place and time, graded by a test such as the Mini-Mental State Examination (Folstein, et al., 1975);

memory evaluated by tests such as a free-recall test of concrete nouns, a 3-4 paired-associated learning test (verbal and nonverbal) by use of a recognition paradigm, the Recognition Span Test (Moss, 1984), and the Brown-Peterson Distractor Test (Peterson and Peterson, 1959; and Armak and Butlers, 1972);

language skills tested by examination of verbal fluency of the semantic or category type, with the examiner writing responses, and by other tests such as the Boston Naming Test (Kaplan, et al., 1978), the Boston Diagnostic Aphasia Examination (Goodlass and Kaplan, 1972), the Western Aphasia Test (Kertesz, 1979), and the Token Test (DeRenzi and Vignolo, 1962; Spellacy and Spreen, 1969) with Reporter's Test (DeReazi and Ferrare, 1978);

praxis evaluated by tests such as those in which the patient copies a drawing (cube, daisy, clock, or house) or performs the block design subtest of the Wechsler Adult Intelligence Scale (Wechsler, 1981);

attention monitored by tests such as a reaction-time task or by the Continuous-Performance Test (Rosvold, et al., 1956);

visual perception studies by use of a variety of tasks, such as the Gollin Incomplete-Pictures Test (Gollin, 1960) and the Hooper Test (Hooper, 1958);

problem-solving skills determined by tests such as the Wisconsin Card Sorting Test (Milner, 1963) or the Poisoned Food Problem Task of Arenberg (Arenberg, 1968); and

social function, activities of daily living, and instrumental activity of daily living, assessed by methods similar to those described in the Philadelphia Geriatrics Center Forms (Lawton, et al., 1982).

100% diagnostic accuracy. The results of these studies however, cannot be generalized since they are small series of highly selected research subjects.

Joachim, et al., (1988) reported a larger series of 150 cases of clinically diagnosed Alzheimer's Disease donated to a brain research program over a three year period. Although diagnosed by over 100 physicians using unknown criteria, 87% of the cases fulfilled histological criteria for AD. While encouraging, these results suggest the relative ease of diagnosing end-stage AD even in the absence of specific criteria. The diagnostic challenge to the clinician and the researcher is to accurately diagnose AD in the early stages of this disease.

**Clinical Studies**

Huff, et al., (1987) studied the utility of cognitive deficits detected by neuropsychological tests for the clinical diagnosis of PROBABLE AD (NINCDS-ADRDA criteria). Of subjects who had 2 or more deficits on testing, 96% had clincally diagnosed AD (96% sensitivity). Of subjects with fewer deficits, 14% had been diagnosed as having AD (86% specificity). Follow-up testing after one year improved sensitivity to 100% and specificity to 89%. Of note, 19% of patients with clinically diagnosed AD had Mini-Mental State Examination (MMSE) scores of 27 or higher, well above the published criteria of below 24 as abnormal (Folstein, et al., 1985). Defining a MMSE score below 27 as abnormal, the sensitivity of the MMSE was 80%; specificity was 95%. Utilizing a cut-off score of 24, sensitivity dropped to 44%. Therefore the MMSE and other mental status tests are relatively insensitive to early cognitive changes.

The patients in this study were felt to be relatively 'early in the disease course' since they were required to give informed consent and be testable neuropsychologically. Twelve percent of patients had only one area of cognitive deficit on neuropsychological examination and would not have met NINCDS-ADRDA criteria. If DSM III criteria requiring memory loss had been used, 4% of patients diagnosed as AD would not have qualified. Hence, the earlier in the course of illness, the less likely they were to meet criteria.

**Reliability Studies**

In a multicenter study, Forette, et al., (1989) studied the reliability of clinical diagnosis using criteria comparable to NINCDS-ADRDA criteria. Diagnoses made at one year intervals were compared and found to have 95% reliability. Although this study did not attempt to establish validity, it showed sufficient reliability to allow comparison of groups at different centers for the purpose of research, including research on the efficacy of pharmacologic treatment.

## Confounders for the Diagnosis of Early AD

### Mental Status Scores/Functional Decline

As noted above, brief cognitive scales can be insensitive to mild degrees of cognitive impairment particularly in well-educated subjects. Although "cut-off" scores for dementia have been recommended for most of these tests, the experienced clinician frequently makes the diagnosis of dementia before these levels have been reached. For example, the published cut-off for the Mini-Mental State Examination (Folstein, et al., 1975) is <24. Most theraputic trials, however, permit entry of subjects with scores of 26 or less, recognizing the potential of patients with early disease to have such high scores. Similarly the Blessed IMC Test generally has a cut-off of >10 errors, but Katzman, et al., (1989) reported individuals with error scores >5 to have a high risk of subsequently developing dementia, suggesting early undetected disease for many of these subjects.

The requirement of the NINCDS-ADRDA criteria for formal testing to document cognitive deficits, while useful, may delay the diagnosis of some individuals. Morris and Fulling (1988) reported a case of early AD with pathological verification in which the majority of the subject's scores were within normal limits. A few scores had shown modest decline over time. One experienced clinician had been unable to detect dementia. Of prime importance in diagnosing this patient was his wife's report of functional decline: forgetting appointments, requiring help on tax preparation for the first time, and getting lost. Similarly, the study of Huff, et al., (1987) detected patients by history who did not yet demonstrate appropriate abnormalities on formal testing. Although the criteria established by the Work Group does not require evidence of functional decline, this information can often be invaluable in making early diagnosis, particularly when there has been no longitudinal follow-up.

### Multiple Areas of Cognition

Dementia is a syndrome of global cognitive impairment; requiring the impairment of at least two areas of cognition would seem appropriate. Numerous patients, however, have been reported initially to have only one affected area such as memory (Neary, et al., 1986), language (Pogacar and Williams, 1984) or visuospatial ability (Crystal, et al., 1982). These individuals do not meet criteria for diagnosis until later in the course of their illness.

### Depression

The relation of depression to dementia is complex. Early in Alzheimer's Disease, up to 1/4 of patients suffer significant depression. Many follow-up series of "pseudodementia" (dementia secondary to depressive symptoms) have reported the subsequent development of Alzheimer's Disease in up to half of the patients (McCallister and Price,

1982; Kral, 1983; and Reding, et al., 1985). The NINCDS-ADRDA criteria allows the diagnosis of AD in the presence of depression, but early in the illness, this distinction can be difficult to make.

### Medications and Concurrent Illnesses

The distinction between dementia and delirium can be difficult to make. Delirium can occur in any person if the metabolic insult is sufficiently great, but older patients are more susceptible to this problem (Katzman, et al., 1988). Relatively minor intercurrent infections, such as urinary tract infections, can produce impaired cognition in the elderly patient. Similarly, toxic exposures (particularly medications) can create conditions easily confused with dementia. The older patient does not metabolize medications as well as their younger counterparts. For example, levels of sleeping medications can build up, leading to a persistant daytime confusion. These situations can present diagnostic difficulty for the clinician.

### Determining AD in the Presence of Cerbrovascular Disease

The clinical diagnosis of mixed AD-multi-infarct dementia is problematic. No validated criteria currently exist for this frequent combination of illnesses. The Hachinski Ischemic Score (Hachinski, 1978) has been valuable in distinguishing AD from vascular dementia but cannot separate individuals with combined disease from the MID category (Molsa, et al., 1985). In the presence of cerebrovascular disease, the diagnosis of Alzheimer's disease cannot be made with reliability.

### Early Dementia and Normal Aging

Perhaps the most difficult challenge in diagnosing dementia is distinguishing the memory and cognitive losses of early dementia from those of normal aging. On the one hand, it is well recognized that changes in memory accompany normal aging. On the other hand, 80% of patients with Alzheimer's Disease have insidious memory loss as their initial symptom. Thus far, these two types of memory loss have been indistinguishable. Longitudinal follow-up of these patients has been the only reliable means of determining the presence of a progressive dementia. Hence, the ability to make a diagnosis improves with multiple assessments demonstrating deterioration of specific cognitive abilities.

## Summary

Clinical investigations of Alzheimer's Disease are dependent on diagnostic accuracy. The criteria developed by the NINCDS-ADRDA Work Group were in response to a need for specific and accurate inclusion criteria to improve the confident identification of subjects with AD. These criteria have contributed greatly by allowing the identification of a

homogeneous group of patients with probable Alzheimer's Disease who can be evaluated in theraputic trials. There are, however, numerous patients with Alzheimer's Disease who do not meet these criteria. Determining the presence of early dementia is a greater challenge than determining the condition producing a dementia. In particular, differentiating early dementia from depression or the memory loss of normal aging remains difficult. A peripheral marker for the diagnosis of early Alzheimer's Disease would be extremely useful and remains a high research priority.

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# Early Diagnosis of Alzheimer Disease

Rodger J. Elble
*Department of Medicine, School of Medicine*
*Southern Illinois University, Springfield, IL*

In 1984, a task force sponsored by the Alzheimer Disease and Related Disorders Association and by the National Institute of Neurological Diseases and Stroke established a set of working criteria for the clinical diagnosis of probable Alzheimer disease (McKhann et al. 1984). With these criteria, the clinical diagnosis of Alzheimer disease (AD) has an accuracy of approximately 85 percent (Wade et al. 1987; Joachim et al. 1988; Katzman et al. 1988a; Tierney et al. 1988). At first glance, this level of accuracy is somewhat surprising, considering the lack of a reliable laboratory test for AD. However, this accuracy is largely a result of the fact that at least 55 percent of all cases of dementia are caused by AD, and there are reasonably reliable tests for the treatable causes of dementia, which account for roughly 15 percent of all cases (Katzman et al. 1988a). Furthermore, many of the other untreatable dementias are readily distinguished from AD, based upon historical and physical findings. However, AD commonly coexists with other causes of dementia, particularly Parkinson disease and vascular disease (Katzman et al. 1988a), and the diagnosis of AD is particularly problematic in patients with mixed disease.

The clinical diagnosis of very early AD is even more problematic. If the ultimate treatment of AD is to be used effectively, clinicians must be equipped with methods of identifying AD at its earliest stages, before disability has reached significant proportions. Detailed neuropsychological testing is useful in the documentation of mild memory and cognitive impairment in patients with questionable dementia, but there are no reliable criteria for predicting which patients will exhibit further deterioration (Rubin et al. 1989; Storandt and Hill 1989). Documentation of the patient's premorbid memory and cognitive performance is very helpful but is seldom available, and the ability of neuropsychological testing to distinguish very early AD from other causes of dementia is limited. Consequently, this communication will review several laboratory methods that could ultimately aid the clinician in the diagnosis of early or even preclinical Alzheimer disease.

## Pathological Diagnosis

A definite diagnosis of AD can be made only by autopsy or by brain biopsy. The neuropathologic criteria are based on counts of plaques and tangles in the neocortex (frontal, temporal, and parietal lobes), hippocampus, and amygdala (Khachaturian 1985). These neuropathologic criteria alone are not sufficient because clinically normal elders may have significant numbers of plaques and tangles in these locations (Ulrich 1982;

Crystal et al. 1988; Katzman et al. 1988b). Consequently, the neuropathologic criteria are supplemented by the requirement that the patient must have a clinical history and examination consistent with AD (McKhann et al. 1984). Furthermore, the required density of plaques and tangles may be revised downwards in a patient with a typical dementia of Alzheimer type (Khachaturian 1985). Thus, the neuropathological diagnosis of AD is both uncertain and somewhat circular. In addition, a critical and unresolved question is the diagnosis of clinically normal elders with sufficient plaques and tangles to satisfy the Khachaturian (1985) criteria for AD (Ulrich 1982; Crystal et al. 1988; Katzman et al. 1988b). The density of neocortical plaques and tangles has only a rough correlation with the degree of dementia (Blessed et al. 1968), and many investigators still question whether existing neurohistological methods are sufficient to fully identify and characterize the neuronal damage in AD (e.g., Davies et al. 1988). These uncertainties in the pathological diagnosis of AD limit the utility of brain biopsy.

A neurosurgeon must restrict biopsy to regions of the brain from which tissue can be safely removed, and this constraint further limits the utility of brain biopsy in the diagnosis of AD. The mesial temporal lobe is avoided because of the risk of memory loss and seizures, and the left frontal and parietal association cortex is avoided because of the risk of aphasia. Clinicians feel most comfortable in taking a biopsy from relatively "silent" areas of the brain such as the nondominant anterolateral temporal cortex and anterior-superior frontal cortex, but biopsies from these areas may not contain diagnostic pathology. Even if sufficient plaques and tangles are found, these biopsies provide no direct information regarding pathology in subcortical grey matter (e.g., substantia nigra, thalamus, basal forebrain), and depending upon the size of the biopsy, significant pathology in the deep cerebral white matter (e.g., Binswanger disease) could be missed. Not surprisingly, cerebral biopsy fails to provide a definite diagnosis in at least 25 percent of cases, and the mortality and morbidity of this procedure is roughly 1 percent and 3 percent, respectively (Sim et al. 1966; Katzman et al. 1988a). The problems of sampling error may be minimized by multiple stereotactic biopsies (Waltregny et al. 1987), but experience with this technique will remain limited until the advent of an effective treatment provides better justification for performing invasive diagnostic procedures.

Neurophysiological Diagnosis

A normal routine electroencephalogram (EEG) is a fairly common occurrence in patients with early AD and is compatible with the diagnosis of probable AD (McKhann et al. 1984). A mild degree of polymorphic theta and delta slowing in one or both frontotemporal areas and disorganization and mild slowing of the background alpha rhythm are also common but nonspecific. Consequently, the general impression among neurologists is that routine electroencephalography lacks sufficient selectivity and sensitivity to be useful in the early diagnosis of AD, but an EEG can be helpful in distinguishing AD from other forms of dementia. For example, a dramatically abnormal EEG in a mildly demented patient should lead to a fervent search for toxic-metabolic, vascular, and infectious etiologies (Markand 1986).

The lack of specificity and selectivity of routine electroencephalography has led to the development of quantitative EEG that employs Fourier analysis to produce a color-coded topographic map of the distribution of activity in the beta, alpha, theta, and delta bands (EEG brain mapping). Topographic mapping has also been applied to the long-latency, event-related potentials that result from auditory and visual stimuli. The general utility of these methods has been hotly debated and has

been the subject of a recent position paper from the American Academy of Neurology (1989). Even the strongest proponents of electrophysiologic studies warn of the limitations and uncertainties of these methods and condemn the practice of interpreting an EEG brain map without a routine EEG and without control data (Duffy 1989).

Quantitative EEG is probably more sensitive that traditional EEG in detecting subtle background slowing and disorganization (Brenner et al. 1988; Nuwer 1989). Although several studies have found differences between Alzheimer patients and controls (Duffy et al. 1984; Brenner et al. 1988; Sheridan et al. 1988), between AD and depressive pseudodementia (Brenner et al. 1988 and 1989), and between AD and MID (Erkinjuntti et al. 1988), these differences are neither sufficiently great nor specific to permit a reliable diagnosis of early AD. The same criticisms are apropos to short- and long-latency evoked-response studies (Harding et al. 1984; Visser et al. 1985; Goodin and Aminoff 1986; Neshige et al. 1988; Pollock et al. 1989). Furthermore, no investigator has demonstrated that quantitative EEG and evoked-response studies are capable of improving the diagnostic accuracy of the more traditional diagnostic clinical studies that are in current use. Thus, there is presently little justification for the routine use of EEG brain mapping and evoked-response studies in the clinical diagnosis of dementia.

## Neuroimaging

### CT and MRI

Gross cerebral atrophy is a generally accepted consequence of Alzheimer disease. The degree of atrophy has been quantified by postmortem examination, by computed tomographic (CT) scanning, and by magnetic resonance imaging (MRI). Atrophy has been carefully quantified in terms of the mass of the entire brain, ventricular volumes, cortical surface areas and volumes, volume and density of subcortical white matter, and the volume of the basal ganglia and other subcortical grey matter (Bird 1982; Albert et al. 1984a and b; de la Monte 1989). Greatest atrophy is seen in the posterior parietal region, amygdala, and hippocampus (de la Monte 1989). This distribution of gross pathology parallels the distribution of plaques and tangles, but the correlation between atrophy and density of plaques and tangles is frequently poor (de la Monte 1989). Furthermore, the degree of atrophy is less than 25 percent in all areas, and there is considerable overlap between Alzheimer and control values (Hubbard and Anderson 1981; de la Monte 1989).

Consequently, numerous authors have found that CT and MRI measures of cerebral atrophy cannot reliably differentiate Alzheimer patients from normal elderly people (Bird 1982; Wilson et al. 1982; Yerby et al. 1985; Luxenberg et al. 1986; Friedland et al. 1988). Thus, while CT and MRI are unquestionably valuable in identifying patients with hydrocephalus, multiple infarcts, subdural hematomas, and tumors (Erkinjuntti et al. 1984; LeMay 1986), these studies are incapable of detecting the presence early Alzheimer dementia. However, the utility of MRI in the diagnosis of early AD may increase as the anatomical resolution of this method improves and as the methods of *in vivo* NMR spectroscopy are developed.

### SPECT, PET, and Xenon Flow Studies

Patients with AD frequently exhibit decreased cerebral blood flow and metabolism in the posterior temporoparietal cerebral cortex, as measured with positron emission tomography (PET), single photon emission computed

tomography (SPECT), and xenon inhalation methods (Jagust et al. 1988; Prohovnik et al. 1988; Waldemar et al. 1988). Initially it was thought that the specificity and selectivity of this finding approached 100 percent (Jagust et al. 1987; Prohovnik et al. 1988), but as more patients were studied, it became clear that the distributions of metabolic reductions may exhibit significant heterogeneity in patients with AD (Friedland et al. 1988; Marc-Vergnes et al. 1989). The estimated specificity and selectivity of these methods are now 75-78 percent (Nagel et al. 1988; Jagust 1989; Growdon 1989). In particular, the utility of these methods in distinguishing AD from vascular dementia is unestablished (Komatani et al. 1988; Waldemar et al. 1988; Jagust 1989). Large prospective longitudinal studies of demented patients, followed to autopsy, are lacking.

These methods of neuroimaging are insensitive to damage in mesial temporal structures (hippocampus and amygdala), which are probably the sites of greatest and most consistent damage in the earliest stages of AD (Terry et al. 1987; Tierney et al. 1988; Zubenko et al. 1989). Consequently, patients may exhibit significant memory loss and exhibit no abnormalities of neocortical blood flow and metabolism on these scans (Cutler et al. 1985; Haxby et al. 1988; Reed et al. 1989). Despite this limitation, PET is capable of detecting decreased metabolism in association neocortex before nonmemory clinical deficits are evident (Haxby et al. 1986). Therefore, while none of these methods can reliably detect the earliest stages of AD, this area of technology continues to advance. With the development of better resolution and more specific tracers, the ultimate utilization of this technology in the detection of very early AD could become a reality.

## Neurochemical Diagnosis

### Neurofibrillary Tangles

A test for the early detection of AD will probably emerge from the ongoing development of assays for elucidating the peptides that comprise neurofibrillary tangles and senile plaques. However, many questions and methodological problems remain.

The component proteins of neurofibrillary tangles have not been completely identified. The presence of tau and ubiquitin are well established (Delacourte and Defossez 1986; Mori et al. 1987; Ihara and Kondo 1989), but these proteins are also found in Pick bodies and in the tangles of normal aged brains, Down syndrome, progressive supranuclear palsy, and the Parkinson-dementia complex of Guam (Bancher et al. 1987; Joachim et al. 1987; Selkoe 1989). Consequently, detection of these proteins in neural tissues and spinal fluid is not specific for AD. It is possible that a different and specific post-translational modification of tau or other filamentous protein could be identified for each of several diseases associated with neurofibrillary tangles, but this possibility is still largely speculative (Goldman and Yen 1986).

Wolozon and coworkers (1986) produced a monoclonal antibody called Alz50 that labels neurofibrillary tangles and neurites in patients with AD. Alz50 reacts with a 68-kDa protein (A68), which may be a form of tau that has been abnormally modified by an unknown post-translational event (Ihara and Kondo 1989; Kosik et al. 1989). Alz50 appears to recognize neurons in the earliest stages of degeneration, before the development of neurofibrillary tangles (Hyman et al. 1988). Trace amounts of A68 are found in Pick bodies and in the perikaryal tangles of the Parkinson-dementia complex of Guam, progressive supranuclear palsy, and normal aging (Wolozin and Davies 1987), but high

concentrations of A68 have been found only in the dystrophic neurites of Alzheimer brains.

Using Alz50 and a Western blot analysis, A68 can been detected in the CSF of Alzheimer victims but not in the CSF of normal elders (Wolozin and Davies 1987). This assay could lead to a useful adjunctive diagnostic test for AD and may also prove particularly helpful in early diagnosis. However, few patients have been studied, and the Alz50 assay for CSF is not quantitative (Davies 1989). Furthermore, not all patients with probable AD have detectable A68 in their CSF. It is unclear whether this failure to detect A68 is due to an error in clinical diagnosis or to a lack of sensitivity of the Alz50 assay. A68 is only relatively specific for AD (Wolozin and Davies 1987), and Alz50 also reacts with 160- to 180-kDa proteins of Alzheimer neurofilaments (Kosik et al. 1989). None of these proteins has been fully characterized. Hence, the specificity and selectivity of this assay are still uncertain.

### Beta Amyloid

The amyloid core of the senile plaques consists of a 4-kDa protein called beta-protein or A4. This protein consists of a sequence of 42-43 amino acids and differs slightly from the meningovascular amyloid protein, which consists of a 39 amino acids (Masters et al. 1985; Prelli et al. 1988; Miller et al. 1989). Both amyloid proteins are fragments of amyloid precursor protein (APP), which is coded by a gene on the long arm of chromosome 21, near the marker for the familial Alzheimer gene (Tanzi et al. 1987). APP is a heterogeneous group of 120-kDa glycosylated proteins that are found in the cell membranes of many neural and non-neural tissues (Selkoe 1989). The amyloidogenic fragmentation of APP could be a primary pathologic event or could be secondary to damage of neuronal membranes (Masters and Beyreuther 1989). Beta-amyloid was recently detected by immunocytochemical techniques in peripheral, non-neural tissues of Alzheimer patients and elderly controls (Joachim et al. 1989). The relationship of this peripheral beta-amyloid to the amyloid of neuritic plaques is not yet clear.

Neurofibrillary tangles and neuritic plaques are occasionally found in the brains of asymptomatic middle-aged and elderly adults (Ulrich 1982; Crystal et al. 1988; Katzman et al. 1988b). The significance of this observation is unclear. The number of plaques and tangles attributable to normal aging is undefined (Crystal et al. 1988). In this regard, high levels of A4 deposition are seen in the brains of patients with no history of dementia. The prevalence of A4 deposition in the brain increases with age much more rapidly than the prevalence of AD that is diagnosed by accepted clinical and neuropathological criteria (Davies et al. 1988). Therefore, the pathophysiologic significance of A4 deposition is still unclear and must be thoroughly understood before an assay of this protein can be used in the diagnosis of preclinical AD.

### Genetic Markers

Only a few families with dominantly inherited AD have been sufficiently studied to develop a DNA marker for the aberrant gene. The gene and its product(s) have not been elucidated in any family. Although the genetic abnormality in a few families has been traced to chromosome 21 (St. George-Hyslop et al. 1987), genetic markers on chromosome 21 have not been found in several other families with early- and late-onset AD (Pericak-Vance et al. 1988; Schellenberg et al. 1988). These observations suggest that the genetic transmission of familial AD is heterogeneous. Furthermore, most cases of AD

are probably sporadic (Farrer et al. 1989). Therefore, DNA markers probably will not achieve general use in the early diagnosis of AD but could prove useful in select families.

### Cholinergic and Other Neurochemical Markers

Alzheimer disease is associated with a deficit in neo- and allocortical cholinergic neurotransmission that is roughly correlated with the degree of neuronal degeneration in the cholinergic basal forebrain (Perry et al. 1982; Whitehouse et al. 1982; Francis et al. 1985; Etienne et al. 1986; Perry 1986; Hansen et al. 1988). Although this deficit is not specific for AD, several groups of investigators have measured reduced levels of acetylcholinesterase (AChE) in the CSF of Alzheimer patients and have examined the utility of these measurements in distinguishing Alzheimer patients from elderly controls. While it is clear that AChE is reduced in the CSF of Alzheimer victims, the variance of measured AChE activity among Alzheimer patients is large, resulting in a broad distribution of values that overlaps significantly with the distribution for normal aging and other diseases (Davis and Goodnick 1983; Nakano et al. 1986; Ruberg et al. 1986, 1987; Appleyard et al. 1987; Elble et al. 1987; Atack et al. 1988; Sirvio et al. 1989).

Choline acetyltransferase has not been consistently measured in human CSF, and measurements of acetylcholine have been as variable as those of cholinesterase (Johns et al. 1983). Elble and coworkers (1989) found that choline was increased in the CSF of Alzheimer victims. However, there was considerable overlap with control values, and this finding is not specific for AD (Elble et al. 1989; Flentge et al. 1989).

Finally, other neurotransmitter and enzymatic abnormalities have been found in Alzheimer CSF. These abnormalities include reduced somatostatin (Tamminga et al. 1987), a negative correlation between serotonin and 5-hydroxyindoleacetic acid as compared to the positive correlation in Parkinson CSF (Volicer et al. 1985), a greater probenecid-induced rise in homovanillic acid as compared to the rise in Parkinson patients (Gibson et al. 1985), and reduced neuron-specific enolase (Cutler et al. 1986). None of these tests is sufficiently sensitive or specific to be used in the early diagnosis of AD.

### Summary

There is still no test that is sufficiently sensitive and specific to permit an accurate early diagnosis of AD. As effective treatments become available, the development of such a test will assume critical importance. This paper has reviewed several lines of investigation that ultimately could prove fruitful. Existing tests must be recognized for what they are: experimental. The routine deployment of any of these tests in the evaluation of dementia is not justified. None of the available laboratory tests contributes more to the diagnosis and management dementia than a thorough and thoughtful clinical evaluation by an experienced clinician. All tests under development must be measured by this standard. It is not enough to simply develop a test that can identify a majority of patients with AD. A truly useful test will also increase the present accuracy of diagnosis and detect the presence of disease before significant pathology has occurred.

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# The Genetics of Alzheimer Disease:
## Identification of Persons at High Risk

Ronald L. Martin
*Department of Psychiatry, School of Medicine*
*University of Kansas, Wichita, KS*

**INTRODUCTION**

Extensive efforts have been made to identify risk factors for Alzheimer's disease (AD). This is for good reason. AD is a dementing disorder associated with major disability and shortened life span. It is also relatively common. Based on current information, the general population risk of AD has been estimated to be over 20% by 90 years of age (Breitner et al., 1988a). A recent community study (although probably diagnostically overinclusive) suggests even higher risks (Evans et al., 1989). The risk of AD escalates dramatically after age 65. The proportion of the population in older age groups is progressively increasing in the developed world. Thus, AD is an increasing public health problem in countries otherwise benefiting from the extension of human survival (Martin, 1989).

To date, the only convincingly demonstrated risk factors for AD are advanced age and positive family history. The latter appears to be via genetic rather than environmental mechanisms (Heyman et al., 1984). Intriguing associations with Down's syndrome (trisomy 21) and ß-amyloid protein, a component of the classic neuropathology of AD and determined genetically on chromosome 21, further implicate genetic risk factors for AD (Selkoe, 1989).

It is likely that the ability to detect AD at incipient stages, and before the illness is symptomatically evident, will be of increasing importance in the future. Recent work suggests that AD may be well-advanced neuropathologically before clinical dementia is clearly evident (Morris et al., 1988a, 1988b). Assuming that effective interventions for AD will be developed, it is likely that initiation of treatment will need to be early in the disease process to be of major benefit. Eventually, interventions may be possible before the disease process becomes established, i.e., in persons predisposed or at "high risk" for AD. It is likely that genetic techniques, particularly identification of an AD genotype or genotypes, will be instrumental in early diagnosis. Characterization of AD genetically would allow the ultimate in early diagnosis since genetic "fingerprinting" for AD could occur even before birth.

Elucidation of the genetics of AD could actually foster development of effective treatments. Isolation of pathologic genes and their associated products could pin-point steps in the AD disease process at which intervention could stall or even reverse disease progression.

Ultimate goals would include prevention or at least postponement of the onset of AD beyond the predisposed person's lifespan.

This chapter will review current knowledge concerning genetic risks for AD, and will outline some directions for future work.

## GENETIC RISK FACTORS

Since some of the very earliest reports, increased risk of AD has been demonstrated repeatedly among relatives of persons with AD (Folstein & Powell, 1984). Such risks, most clearly apparent in some striking pedigrees, has also been observed in studies of series of patients (Martin, 1989). Twin studies have been scanty and evidence from them is provocative but somewhat conflicting. Further evidence derives from recent molecular genetic findings. The latter suggest that fundamental discoveries may soon be at hand which will lead to a basic understanding of the genetics of AD. The following sections will review such findings.

### *Family genetic studies*

Within 20 years of the identification of AD as a clinicopathologic entity, studies by Meggendorfer (1926) and Weinberger (1926), although flawed by probable inclusion of probands with other dementing disorders, suggested that relatives of patients with "senile dementia" were at increased risk for AD. They also noted striking pedigrees in which the disorder appeared to be transmitted directly over several generations.

The increased risk suggested by these early studies was supported by a number of studies of both presenile "Alzheimer's disease" and "senile dementia" over the next four decades (Sjogren et al., 1952; Wheelan, 1959; Larsson et al., 1963; Feldman et al., 1963; Bucci, 1963; Constantinidis, 1965; and Akesson, 1969). Excellent reviews of these studies are available (Folstein & Powell, 1984; Breitner, 1987).

The studies of presenile "Alzheimer's disease" by Sjogren et al. (1952), and of "senile dementia" by Larsson et al. (1963) deserve special mention in light of their considerable influence. These studies differentiated "Alzheimer's disease" and "senile dementia" primarily on the basis of age at onset, but also on the basis of the presence or absence of certain clinical signs. "Alzheimer's disease" was defined as a presenile dementia with an onset roughly prior to the age of 70 and with focal signs and aphasia. "Senile dementia" had a later onset and an absence of focal signs and aphasia.

Considering the Sjogren et al. (1952) and Larsson et al. (1963) reports together, the following major conclusions were made:

1). The risk of dementia to first degree relatives of "Alzheimer's disease" probands was about 10% and suggested a multifactorial mode of transmission;

2) The risk of "senile" dementia to first degree relatives of affected probands was about 23% by age 90. Risks to first degree relatives were 4.3 times risks in the general population at corresponding age points. The frequency of a "senile dementia" gene in the general population was calculated as 6%. "Senile dementia" was transmitted by an autosomal dominant gene with an estimated penetrance of approximately 50%. Thus, 12% of the population (homozygotes plus heterozygotes) would carry an affected gene. This group would be at 50% genetic risk for "senile dementia;"

3). Risks were specific, i.e., the risk of "senile dementia" was not increased among relatives of presenile "Alzheimer's" cases and the risk of presenile "Alzheimer" dementia was

not elevated among relatives of the "senile dementia" cases.

However, a number of methodological shortcomings, many deriving from the assumption of two separate illnesses, call several of these conclusions into question.

In the Sjogren et al. (1952) study, relatives of presenile "Alzheimer" cases older than 70 years of age were not examined for dementia. Thus, relatives were only observed through a portion of the total risk period for "Alzheimer's disease"/"senile dementia," considered as a composite. This restriction probably contributed to the rather low estimated risk to first degree relatives (10%), an estimate which seemed to justify the conclusion of a multifactorial etiology for "Alzheimer's disease" and the exclusion of a major gene effect.

In the Larsson et al. (1963) "senile dementia" study, most probands were selected by record review and included subjects with evidence of multi-infarct disease. Reviewing the case descriptions, Breitner et al. (1986a) estimated that at least one-quarter would not have received a diagnosis of AD according to criteria used in AD research today. The probable inclusion of nonAD cases in the proband sample would dilute elevated genetic risks in the families of actual AD cases. In addition, reliance (although only in a minority of families) upon parish registry data rather than field study would contribute to underdetection of secondary cases (affected relatives). These factors would contribute to underestimation of risk.

The evidence for the genetic independence of "Alzheimer's disease" and "senile dementia" was highly circular. In the Sjogren et al. (1952) study no secondary "senile dementia" cases were reported among relatives of "Alzheimer's disease" probands. However, as mentioned above, relatives over 70 were not evaluated for dementia. In the Larsson et al. (1963) study no presenile "Alzheimer's" cases were reported in relatives of the "senile dementia" probands. Yet, secondary cases of "senile dementia" with symptomatic onset as early as at 52 years of age are described (Larsson et al., 1963, p. 64). This illustrates the bias which can be introduced into a generally rigorous study by preconceived assumptions about disease boundaries.

Assumption of two separate illnesses, differentiable according to age at onset (presenile "Alzheimer's disease" vs. "senile dementia") has a long tradition. It can be traced to the earliest reports. In the original case identified by Alois Alzheimer, initial symptoms were noted at age 51 and the illness progressed rapidly with the patient dying in a severely debilitated, demented state within five years of the apparent onset (Alzheimer, 1907). Dementia in the senium was well known and was attributed primarily to the aging process itself. It was the early age at onset and the finding of neuropathological changes previously thought to occur only in much older demented patients which made this case notable (Torack, 1978).

Dichotomization of AD is now challenged since cases at both ends of the age spectrum are characterized by the same types of neuropathological changes (Martin, 1989). While further discussion of the importance of age at onset will appear later, in the remainder of this chapter *Alzheimer's disease (AD)* will refer to presenile "Alzheimer's disease" and "senile dementia" in the composite.

Another major series of studies was that of Heston and colleagues (Heston, 1977, Heston & Mastri, 1977, Heston & White; 1978, Heston et al.; 1981, Heston & Mastri, 1982, Heston, 1984, Cutler et al.; 1985). Neuropathologically verified cases from the Minnesota state hospitals were utilized. Presenile cases comprised the initial sample with senile cases added later.

Heston found that morbid risks to first degree relatives of presenile cases were elevated over controls (Heston et al., 1981). The risk to siblings of probands with an age at onset before age 70 and an affected parent approached 50% by age 85. Risks to first degree relatives of late onset cases were not elevated as compared with risks to relatives of controls. This could be interpreted to represent demarcation into two illnesses: "familial, early onset", and "nonfamilial, late onset" disorders, a scheme reminiscent of the "Alzheimer's disease"/"senile dementia" dichotomy of Sjogren and Larsson.

However, such an interpretation should be considered cautiously. Heston demonstrated correlation of age at onset within families (Heston et al., 1981). Thus, affected relatives of late onset probands would tend to have late ages at onset as compared with affected relatives of early onset probands. As such, they would be more likely than those of early onset probands to die of other causes before ascertained as affected. In addition, it would be more likely that mild cognitive decline representing early AD would be considered an aspect of "normal" aging in late onset cases. Both of these factors would contribute to incomplete ascertainment of secondary cases and an underestimation of risk for relatives of late onset probands. As a result, late onset illness would appear to be nonfamilial.

Another interesting aspect of the Heston studies includes the determination of increased risk of Down's syndrome and myeloproliferative disorders among relatives of AD probands (Heston, 1977, Heston, 1984). Heston posited that a common defect in the organization of microfilaments and microtubules underlies these conditions as well as AD (Heston & White, 1978). Although not consistently supported in other studies, the association with Down's syndrome gave impetus to a search for an AD gene on chromosome 21.

Other investigations have implicated genetic risk factors for AD but have not resolved the many questions surrounding the nature of this risk, including whether or not AD can be dichotomized into familial and nonfamilial types.

Whalley and colleagues found an excess of dementia among first-degree relatives in a study conducted in Scotland of AD probands with an age at onset prior to age 65 (Whalley et al., 1982; Wright et al., 1984). They observed four secondary cases (four others were excluded despite death certificate diagnoses suggesting AD). They concluded that the observed magnitude of increased risk approximated the square root of the estimated population incidence of AD, suggesting a "simple polygenic model." As in the Sjogren et al. (1959) study, very few persons were observed past the age of 69 eliminating from ascertainment the major period of risk.

Heyman et al. (1983, 1984), in the Duke collaborative studies, only included probands with a symptomatic onset of AD under 70 years of age. The risk of AD to first degree relatives was 14% by age 75. Mental changes first appearing after age 75 were not considered because of the "difficulty of making a diagnosis of Alzheimer's disease on the basis of previous clinical records in persons older than age 75." Again, this would be expected to result in an underestimation of risk.

Heyman et al. (1983) concluded that the majority (75%) of probands had nonfamilial illness but that a single autosomal dominant gene could be operating in the 25% of probands with pedigrees with multiple cases ("familial AD"). It was acknowledged that some of the nonfamilial cases would have proved familial if other relatives "had survived or had been observed long enough to develop overt symptoms, or if neuropathological examination had been carried-out." Over half of the secondary cases occurred in the "senile" period between

ages 66 and 75. This contradicted Sjogren's and Larsson's conclusions about genetic independence of presenile "Alzheimer's disease" and "senile dementia."

Recent studies by Mohs et al. (1987), Huff et al. (1988), Martin et al. (1988), and Zubenko et al. (1988a) reported risks approximating 50% to first-degree relatives of both early and late onset cases by late in the ninth decade. Age at onset was correlated in the Mohs, Huff, and Zubenko studies. Zubenko et al. (1988a, 1988b) made the additional observation that patients with an early onset tended to have increased platelet membrane fluidity and might represent a subgroup even though they were no more or less familial than patients with a later age at onset.

Breitner and Folstein (1982, 1984) reported that a familial form of AD can be differentiated from nonfamilial forms on the basis of the presence of aphasia or apraxia in addition to the usual amnesia during the middle stages of AD. A lifetime risk of 52% was observed in relatives of aphasia-apraxia cases while risks were not elevated among relatives of nonaphasic probands in this series. The aphasia-ataxia dichotomization was based on whether or not a subject could write a meaningful sentence of their own creation. It was argued that ascertainment of aphasia by the sentence writing procedure was appropriate since results were highly correlated with aphasia-apraxia assessed by "more costly" methods.

Differentiation of familial and nonfamilial illness on the basis of aphasia has not been supported by other studies. Heyman et al. (1983) found equal risks to relatives of probands with relatively normal vs. abnormal language scores, as did Cummings et al. (1985). Knesevich et al. (1985) reported greater risks to relatives of nonaphasic compared with relatives of aphasic probands.

Breitner (1985) comments that contrasting findings may be attributable to various methodological differences in the studies including the stage of AD in probands (mild in the Knesevich et al. (1985) study, more severe in the Breitner & Folstein study). Methods of ascertaining aphasia may also make a difference. Knesevich et al. used global ratings from a modification of the Boston Diagnostic Aphasia Examination, while Breitner & Folstein used the more simple method described above.

The previously mentioned Mohs et al. (1987) study, as well as a study reported by Cummings et al. (1985), call into question the utility of differentiating familial from nonfamilial cases on the basis of aphasia because of the virtual universality of such symptoms in AD patients. In the Mohs study, 94% showed aphasic signs. Cummings et al. (1985) argued that aphasia was so constant a feature of AD that diagnosis of AD without aphasia should be discouraged. Such a common characteristic would not be expected to be a useful discriminator.

Thus, it is not clearly possible to identify familial and nonfamilial forms of AD on the basis of early onset, aphasia-apraxia, or any other factors. A lifetime risk approximating 50% with survival to 90 years of age or so (this requires careful age-correction procedures) has been found in a number of recent studies. Such findings are at least consistent with a model suggested by Chase et al. (1983), positing autosomal dominant transmission with complete, but age dependent penetrance. In this view, the lifetime risk to first degree relatives of AD cases would approximate 50%, but this would only be achieved at the end of the risk period which extends at least to the end of the ninth decade. Thus, establishing segregation ratios, which relate to estimated risks, requires appropriate life table and survival analysis methods.

*Difficulties with family genetic studies AD*

While it is well-accepted that genetic factors determine increased risk for at least some types of AD, debate continues about the mechanism of transmission. This is in distinction to the situation with Huntington's disease (HD) for which autosomal dominant transmission has been long-accepted (Slater & Cowie, 1971). Recently, linkage of an HD gene to a segment of chromosome four was established (Brandt et al., 1989).

Controversy about the genetics of AD may result from greater genetic complexity of AD relative to HD (for example if AD proves to be genetically heterogenous). Alternatively, the mechanism of genetic transmission for AD may be relatively simple, but may remain obscure largely because of AD's late age at onset (Breitner et al., 1986b, 1988b). The risk curve for AD is shifted to later ages by perhaps 30 years relative to the curve for HD. Based on existing evidence, a "theoretical" risk curve has been proposed with a risk distribution (based on age at onset) peaking at age 81, with 95% of the risk compressed between 70 and 90 years of age (Breitner et al., 1988a). Risks of developing AD are very low under the age of 70 years, increase progressively to an asymptote at age 81, then progressively decline with little risk remaining after 90 years of age.

Actually, the peak observed incidence of AD occurs at a point several years younger than this theoretical peak (Breitner et al., 1988a). This may be attributable to the fact that the mean age at onset for AD occurs later than the current mean age of survival. As reviewed by Breitner et al. (1988a), recent census data show a mean life expectancy in the US between 75 and 80 years of age with perhaps 1% surviving to 90 years of age. Assuming that the Breitner et al. risk curve for AD is accurate, very few persons will live through the full period of risk. Thus, even if an autosomal dominant mechanism is involved in the transmission of AD, the risk to relatives is considerably less than what would be expected for a disease such as HD with an earlier age at onset (Breitner et al., 1988a). Many will succumb to various causes of death unrelated to AD before the genotype manifests phenotypically.

In other words, manifestation of the underlying genetic risk is "censored" by mortality from unrelated causes (Chase et al., 1983). A person afflicted with AD may have no affected relatives known. They will appear to suffer from a nonfamilial form of the disease simply because affected relatives may not have lived long enough to manifest the AD genotype (Chase et al., 1983).

Thus, a minority of first degree relatives who carry an AD genotype will actually manifest the disorder. Rigorous age adjustment statistics can be employed and observed risks to first degree relatives may approximate the 50% expected for an autosomal dominant trait. Yet, the inability to completely ascertain affected relatives limits the power of observation.

This situation will not change in a major way even with extension of the human lifespan. It has been hypothesized by Fries (1980) that there are inherent limits to the human lifespan even with successful treatment of acute diseases and elimination of risk factors for many chronic diseases. Fries argues that a species-specific natural "senescence" or age-determined decline exists which limits human survival even under the best of circumstances. With exclusion of "preventable" causes of death, "theoretical" human survival is normally distributed about age 85 years of age, with 95% of the non-preventable deaths occurring between age 77 and 93 years of age. Thus, not only does the risk curve for AD exceed the observed incidence curve for AD, it may closely approach the theoretical limit of the human lifespan.

In addition, the late age at onset limits the possibility of prospective, multigenerational studies. More practical studies combining examination of surviving relatives and utilization of

records of deceased relatives also have difficulties. Symptoms in affected relatives may not have developed or progressed far enough at the time of study to indicate the diagnosis of AD.

Diagnostic problems must also be considered. At advanced age intellectual decline resulting from AD may be attributed to "normal" aging, so-called "benign senescent forgetfulness" (Martin, 1989). With such decline, memory problems are not clearly incapacitating and are said to represent lack of memory for "part" but not the whole of an experience.

The validity of this designation has recently been called into question. In a study by Martin et al. (1988), relatives with questionable dementia corresponding roughly to this definition were observed among relatives of AD probands but not among relatives of controls. Morris et al. (1988b) reported that five subjects with "questionable" dementia (essentially mild memory problems in the absence of other intellectual decline) showed neuropathologic changes typical of fully developed AD on autopsy. Only one had progressed symptomatically to definite dementia by the time of death. At present, a total of nine have gone to post-mortem (Morris, personal communication). Neuropathological diagnosis was definite in all nine, yet only in five had symptoms progressed enough to warrant a definite dementia diagnosis. This suggests that the AD genotype may express itself on a neuropathological basis well before, and even in the absence of the clear development of symptoms. Such phenotypic variability among affected individuals may confound genetic analyses.

Other methodological problems exist. As demonstrated by Breitner & Magruder-Habib (in press) even conventions used to determine the age at onset among probands and secondary cases can influence familial risk estimates. For example, if the age at onset for probands is equated with the age at the onset of symptoms, and the age at onset for secondary cases is the age at which a progressive AD-like dementia is definite, familial risks will be seriously underestimated.

Returning again to the controversy concerning the differentiation of early and late onset illnesses, Breitner et al. (1988a) acknowledge a "toe" of greater than expected incidence of early onset disease. A mixed distribution suggests genetic heterogeneity, a situation which would greatly confound simple genetic analyses. There is also the possibility of "phenocopies," i.e., occurrence of cases with similar or identical appearance to AD but with nongenetic etiologies.

Resolution of many of these problems would be greatly aided by the identification of biological markers as described below.

*Search for biological markers*

The preceding discussion suggests that family genetic studies utilizing diagnoses based on symptomatic presentation alone will never fully elucidate genetic risks. Recent work suggests the possibility of identifying biological markers in nonbrain tissues. Talamo (1989) found abnormal masses of neurites in neuronal elements of sensory epithelium in 8 of 9 AD patients but in only 2 of 14 age-matched controls. The masses resembled changes seen in AD with similar composition of subunits, and reaction to two antibodies (ALZ50 and a monoclonal antibody to tau protein). These antibodies specifically stain pathological structures in AD. How early in the disorder such changes can be detected is unknown and awaits further study. Also of interest is the report by Joachim et al. (1989) of the detection of greater proportions of amyloid ß-protein deposits in non-neural tissues (including blood vessels, skin, subcutaneous tissues, and intestine) in AD vs. nonAD aged persons. If replicated and refined, such methods would allow relatively noninvasive antemortem ascertainment in questionable

cases.

The ultimate biological marker would be determination of the AD genotype or genotypes. Ultimately, this would allow deoxyribose nucleic acid (DNA) diagnosis. The genetics of AD may well turn-out to be extremely complex. Yet, genetic marker techniques would allow more definitive testing of potential mechanisms of genetic transmission.

The search for genetic markers involves the use of molecular genetics, a newly emerging technology. Such techniques have been instrumental in closing-in on the Huntington's disease (HD) genotype (Brandt et al., 1989). Applications of molecular genetics to the study of AD will be the focus of a later section of this chapter.

*Twin studies*

Twin studies represent another classic approach to the elucidation of genetic risks of disease. Concordance observed in monozygotic (MZ) twins is compared to rates in dizygotic (DZ) twins. Interpretation hinges on the assumption that MZ and DZ twins are exposed to the same nongenetic influences, while only the MZ twins are identical genetically. For a disease transmitted genetically, greater concordance rates would be expected for MZ twins than for DZ twins. For a disease that was purely genetic, concordance would be 100% if both lived through the full period of risk.

Twin studies of AD have been reviewed succinctly by Resnick et al (1988). Anecdotal reports include at least five concordant and four discordant MZ twin pairs. Two patient series have also been reported. Kallmann (1956) found 42.8% concordance for MZ twin pairs and 8.0% for DZ twins. Jarvik et al. (1980) studied the same patients at a later time, and found additional concordant cases among the MZ twin pairs. The Kallmann data is compromised by diagnostic criteria which are not up to current standards. In a more recent series, Nee et al. (1987) reported 7 of 15 MZ and 2 of 7 DZ twin pairs concordant for AD.

Interpretation of AD twin data is complicated by several factors. As in twin studies of any illness, an ascertainment bias exists for selection of concordant pairs for study. This favors estimation of greater concordance than is actually present. Particularly in older studies, inaccuracy in establishing zygosity (whether twins are MZ or DZ) may have compromised valid conclusions.

Twin studies of AD involve several additional problems. Longevity is more highly correlated in MZ relative to DZ twin pairs. In a disorder with a late age at onset such as AD this favors more similar exposure to risk among MZ twin pairs than among DZ twin pairs. MZ twin pairs are more likely to be bias in favor of concordants than DZ twin pairs on this basis alone. Other factors could bias towards nonconcordance. These include variability in age at onset, persistence of the risk period into very late age when dementia from other causes is more likely, lack of a clear endpoint of risk, and the need for neuropathologic verification for definitive diagnosis.

Findings in a recently reported concordant MZ pair (Resnick et al., 1988) may have important implications for understanding the interplay of genetic predisposition and manifestation as disease. Both twins manifested AD yet they showed marked metabolic, structural, and behavioral differences. Although similar ages at onset were apparent, one twin was at a much more advanced stage of disease at the time of study. The more severely impaired twin had bilateral deficits in tactile functions and a left hemi-attention. The other twin had more right-sided sensorimotor deficits. Differences in regional metabolism paralleled these behavioral patterns. As will be discussed in the following section, such phenotypic

variability in the expression of the same genotype make twin, and other genetic studies of AD more difficult.

*Molecular genetic studies*

Characterization of a defective AD genotype would allow definitive identification of those at high genetic risk for AD. This remained a remote possibility before the advent of molecular genetic technology. This technology promises the eventual localization, characterization, cloning, and even modification of genes responsible for a variety of inherited disorders including AD.

Before development of molecular genetic techniques, direct study of the human genome was limited to karyotypic examination for chromosomal anomalies or aneuploidy, or a search for linkage of AD with the limited number of known physical or biochemical chromosomal markers. Early reports of the association (at least in women) of hypodiploidy with AD (Nielsen et al., 1970) were not supported by subsequent studies (Moorhead & Heyman, 1983).

Linkage studies were also not productive. Failure to establish linkage is not surprising. Linkage represents close proximity of a defective gene to a known marker. With an estimated 20 million gene locations on the human genome and a very limited numbers of markers, the probability of such localization was remote.

Molecular genetic techniques allow creation of innumerable DNA markers by cleaving DNA using restriction endonucleases. The resulting DNA fragments, also known as restriction fragment length polymorphisms (RFLPs), can then be used as markers in linkage analyses. Since RFLPs of different lengths from any chromosome can be produced, microdissection of the human genome is possible. More extensive discussion of such techniques are reviewed in another chapter of this book (Wizniewski, 1989).

Molecular genetic strategies have proved successful in ascribing defective genes to specific chromosomal regions; and even to specific loci, for a number of medical conditions including cystic fibrosis and polycystic kidney disease (Motulsky, 1989). The Committee on the Mapping and Sequencing of the Human Genome, newly instituted by the National Research Council, represents a Herculean effort with a goal of mapping the human genome. The bulk of this work is to be accomplished within the next five years. Resulting discoveries would include identification of genetic mechanisms contributing to many diseases including neuropsychiatric disorders such as AD (Olson et al., 1989).

Among neuropsychiatric disorders, findings have been conflicting regarding the location of genes associated with schizophrenia and manic-depressive disorder (Barnes, 1989). On the other-hand, localization to particular chromosomes have been established for Huntington's disease (Brandt et al., 1989), von Recklinghausen neurofibromatosis (Fountain et al., 1989), and the adult $G_{M2}$ gangliosidosis form of Tay-Sachs disease (Navon & Proia, 1989). Leads exist as to characterization of the responsible genetic defects and the associated biochemical differences in these conditions.

Chromosome 21 appeared to be a good place to search for a responsible genetic defect for AD (Heston, 1984; Selkoe, 1989). Chromosome 21 was implicated in several ways. First of all, virtually all Down's syndrome (trisomy 21) patients surviving into their forties, develop AD-like neuropathological changes, although not necessarily a clinical dementia. Down's syndrome occurs only with duplication of chromosomal material from an "obligate" portion of the proximal section of the long arm of chromosome 21.

Culpability of chromosome 21 was boosted by the observation of Heston (1977) of an excess incidence of Down's syndrome in the relatives of AD patients. Heston also reported an excess of myeloproliferative disorders (also in excess among Down's syndrome patients), and an increase in miscarriages and/or infertility among relatives of AD patients. He hypothesized that the association of these various conditions could be explained on the basis of a defect in microtubules, this defect common to all of these disorders (Heston & White, 1978).

Indeed, a linkage-analysis reported by St George-Hyslop et al (1987a) of four families in which autosomal dominant transmission of AD was apparent over several generations indicated linkage with two chromosome 21 probes (D21S1/D21S11 and D21S16), localized to the q21 region of chromosome 21. In this study, an early age at onset of AD was characteristic for affected relatives. Intriguingly, a gene for ß-amyloid protein was localized in the same region (q21) of chromosome 21 as the AD gene (Tanzi et al., 1987a).

ß-amyloid protein is found in excess in the brains of Down's syndrome and AD patients and is intimately associated with neuritic plaques (Robakis et al., 1987, and perhaps with neurofibrillary tangles (Kang et al., 1987). A preliminary study suggested that AD subjects had an "extra dosage" of the ß-amyloid gene (Delaber et al., 1987). The researcher hypothesized that the AD and ß-amyloid genomes were identical, and that genetic duplication of a subsection of chromosome 21 resulted in over-production of the corresponding protein, which resulted in AD.

At that point it appeared that a fundamental breakthrough had been made. However, subsequent studies have indicated that things are not that simple. Tanzi et al. (1987b), following-up on the preliminary Delaber study with a more complete investigation, demonstrated that the critical segment of chromosome 21 was not duplicated. Lack of duplication has been reported by others as well (Podlisny et al., 1987; St. George-Hyslop, et al., 1987b; ): Other reports indicated that the genetic defect for AD was not tightly linked to the ß-amyloid gene (Tanzi et al., 1987c; Broeckhoven et al., 1987). Two other studies do not support linkage to the chromosome 21 probes (Schellenberg et al., 1988; Roses et al., 1988).

Schellenberg et al. (1988) studied linkage in five families in which AD was transmitted over several generations in a manner consistent with an autosomal dominant pattern. More general findings were reported previously by Bird et al. (1988). AD was not linked to chromosome 21 markers in this group. This suggests the existence of an AD gene which is not on chromosome 21 at least in these families. Interestingly, the families all derived from a geographically and genetically isolated group of Volga Germans. This suggests that their disease may have derived from one common ancestor, a so-called "founder effect". AD would most likely represent a genetically homogenous disease in this group.

Roses et al. (1988) reported on the study of 10 AD families with apparent autosomal dominant transmission. This study also did not indicate linkage to chromosome 21 loci. Affected individuals in both the Volga German and the Roses et al. studies had relatively late ages at onset compared with the St. George-Hyslop study. This would support an interpretation that at least two genetic forms of AD exist: an early onset form, perhaps determined by an autosomal dominant gene on chromosome 21; and a late onset form, determined by a gene with similar autosomal dominance but at a different location.

It must be remembered that conclusions about a chromosomal or genetic definition of AD at this time are premature. Linkage studies by nature yield probabilistic not absolute findings. When based on relatively small numbers of cases, even a misdiagnosed case or two

can distort the linkage probabilities (lod scores). It has been suggested that the failure of a subsequent study to replicate an earlier determination of linkage of manic-depressive disease to chromosome 11 may be attributable to such effects (Barnes, 1989). The same may be the case with AD.

It must be remembered that even if linkage studies establish a chromosomal location for AD, this would only allow genetic diagnosis within a particular informative family. To be informative, chromosomal material must be available from multiple family members including the affected relative. Definitive identification of a person at risk for AD would require characterization of the actual defective gene. If AD turns-out to be genetically heterogenous this task will be extremely difficult.

## APPLICATIONS OF GENETIC IDENTIFICATION OF HIGH RISK

### *Research*

*Genetic research.* The inability to identify persons who will eventually develop AD should they live long enough has greatly hampered genetic research. As previous sections attest, a fundamental problem in AD genetic research involves the late and symptomatically variable onset of AD. Assuming that at least some cases of AD are genetically determined, it is not now possible to discern whether or not an individual not manifesting AD clinically at the time of death or ascertainment carries an affected genotype. Statistical age and risk corrections may adjust for this. Yet, another source of uncertainty is introduced. Molecular genetic characterization of an AD genotype or genotypes predisposing to AD would circumvent such difficulties.

Molecular genetic understanding would also enable researchers to determine more definitively a number of unsolved questions concerning AD. If at least some AD cases are genetically determined, do these represent a genetic unity or do multiple predisposing genotypes exist? Do nongenetic or environmentally derived (so-called phenocopies or sporadic) subtypes exist? At what frequency do unexpressed genotypes occur? Why is age at onset so delayed in some cases relative to others? Are there environmental factors which contribute to the expression or nonexpression of an AD genotype?

*Treatment research.* Ability to identify individuals at high genetic risk for AD would greatly aid in the development of effective treatments. With characterization of a predisposing genetic defect, much could be learned about the pathophysiology of AD, allowing a rational approach to treatment research. As Davies (1988) said, AD..."is a complex disease. It probably represents a cascade of processes which ultimately produce the disease state. Effective intervention is possible when, from understanding the basic underlying disease process, crucial interventions can be made reversing or neutralizing this cascade."

Once a defective gene is identified, characterization of a gene product could follow. It might then be possible to institute measures to eliminate or at least limit expression of the gene product. In general, the action of a gene involves synthesis of a protein. In a disease state this synthesis may be in some way defective. Thus, the protein could be deleted (which would require replacement), under-produced (which would require supplementation), over-produced (which would require limiting production), or produced in an aberrant form (which might require blocking or in some way neutralizing production of the aberrant form and substituting the necessary form).

As discussed previously, the risk curve for AD approximates the actual and even theoretical human survival curves. Thus, effective treatment may not require total correction of a defect we genotype, but merely postponement of its expression until beyond the person's

lifespan.

Even more innovative interventions would include direct manipulation of the genes themselves, so-called "human gene therapy." As recently reviewed by Friedmann (1989), potential strategies include gene replacement, correction, and augmentation. Such techniques would involve the removal, replacement, or alteration of a specific sequence, or modification of the expression of a pathologic gene by introducing foreign DNA. Potentially, gene transfer could be accomplished using physical, chemical, or viral vector methods.

Gene therapy has been suggested for several diseases including various blood dyscrasias and cystic fibrosis. The former are perhaps ideal in that it may be possible to implant genetically modified cells at the site of the relevant disease (the bone marrow) where they could replicate (Friedmann, 1989). Such strategies have several problems when it comes to diseases of the central nervous system (CNS). CNS cells are more difficult to access. The presumed target cells, neurons, are post-mitotic and relatively refractory to alteration of genetic sequences.

In the case of AD, one promising finding is that retrovirally transduced rat fibroblasts are capable of producing nerve growth factor or NGF (Ad Hoc Working Group, 1989). NGF was found to protect cholinergic neurons from degenerating in rat brain after lesions to the fimbria fornix. NGF has been observed to have similar effects in AD preparations. This is especially intriguing since the cholinergic neurotransmitter system degenerates disproportionately relative to other CNS systems early in the course of AD.

If early, even prenatal, detection of those genetically predisposed to AD were possible, intervention could occur before the disease was established. As previously mentioned, recent research indicates that neuropathological changes associated with the disease may be already firmly established by the time the person shows even equivocal clinical manifestations of AD (Morris et al., 1988a, 1988b). At such time it may be too late to reverse, or even prevent progression of the disease process.

*Current clinical applications*

Currently, practical applications of identifying those at high risk are limited to promotion of early diagnosis, psychosocial planning, and genetic counseling.

*Accurate diagnosis.* Knowing a person is at high risk for AD could facilitate early diagnosis. The time from the onset of AD symptoms to first seeking evaluation and treatment is often prolonged. Knowing that a person is at high risk could sensitize family and care-givers to the onset of even nonspecific symptoms. Early changes with AD include not only memory impairments but often depressive symptoms (Knesevich et al., 1983), psychotic symptoms (Rubin et al., 1989), and even personality changes. Such symptoms are often quite amenable to treatment (Martin, 1989). Such interventions will not alter the course of dementia, but may considerably improve the quality of life for the patient and care-givers.

Experience with Huntington's disease (HD) may bear on such concerns. It is now possible to identify those carrying the defective HD gene with a high degree of certainty (Brandt et al., 1989). A pilot study of the effects of informing persons of whether or not they are at risk revealed no increase in suicide or in the rate of observed depression among those informed. This of course does not mean that such information should be given casually (and this was not the case in the HD study), but with proper cautions it can done safely and productively.

*Psychosocial planning.* Another advantage of early diagnosis is the fostering of early psychosocial planning. Such planning can be of great benefit to the patient and care-givers (Martin, 1989). In addition to promoting early anticipation of the need for placement or other supervised care, it would allow more effective occupational planning and disposition of assets before the person became incapacitated to a major extent. It would also allow exploration of the patient's wishes concerning the level of medical intervention should a life-threatening situation arise (Martin, 1989). If a person were identified as affected with an inevitable risk of AD this question could be explored before major incapacitation had occurred.

*Genetic counseling.* For disorders such as AD with age of onset in late adulthood, genetic counseling entails complexities in addition to those encountered with conditions clearly evident in early childhood or at birth (Heston, 1976). In the case of early onset disorders, most questions involve risks to young, unborn, or potential children. With late onset conditions, adult relatives also have concerns about their own risk. "My father (or mother, grandfather, grandmother, brother, sister, uncle, aunt, etc.) has Alzheimer's disease. What is my risk?"

Answers to such questions are first of all complicated by the fact that a genetic mechanism for the transmission of AD is not established. Even assuming autosomal dominant transmission with age-dependent penetrance, the problem of potential phenocopies or nongenetic cases exist.

All dementia is not AD. For example, if dementia in the relative of a concerned person was the result of a different degenerative disorder, multiple cerebral infarctions, infection, or tumor, that person would have an AD risk no different from an otherwise comparable person in the general population. For a demented relative who died remotely and without careful neuropathological investigation, it may be impossible to establish a diagnosis with any authority. Even for a living symptomatic relative with a complete antemortem evaluation short of brain biopsy, an AD diagnosis can be made with only 80% accuracy (McKhann et al., 1984). Greater certainty may be possible if DNA diagnosis of AD is developed, but such techniques are not now available.

If it becomes possible to identify genotypes which will result in AD should the person live long enough, what will be the effect of an individual learning of such predisposition? Would it not be better to remain unaware of such a devastating potential, considering that at present such knowledge would not lead to effective treatment? Will such knowledge lead to an increase in suicide or depression?

In considering risk to potential children of a person whose own parent develops AD an additional layer of uncertainty is added. Even if it were established that the person's parent was affected with AD, the genotype of the potential parent will generally remain unknown until after child-bearing years. Thus, genetic counseling may have little relevance to the decision of whether or not to have children. Again, discovery of a genetic marker allowing linkage studies or even direct genetic diagnosis would greatly diminish such uncertainty.

Even if technologies making accurate diagnosis and definitive determination of risk are developed, the implications of risk for AD are different than for disorders with age at onset earlier in life. In a sense, AD is a less incapacitating disorder (Tsuang, 1978). After all, in most cases an affected AD genotype will not be expressed until near the end of an otherwise unaffected lifespan. As previously discussed, most persons with an affected genotype will die of unrelated causes before they experience any symptoms of the disorder, let alone major impairment. Even leaving ethical or religious concerns aside, it is doubtful that many would

forgo procreation or choose abortion for a disorder with this time course.

Given the above limitations, genetic counseling for AD should be performed very cautiously and tentatively (Pardes et al., 1989). With such an attitude, coupled with guidance in interpreting the best available information about genetic determination of AD, genetic counseling can be effective in dispelling mistaken notions and excessive and even needless concerns about genetic risks for AD.

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# Early Identification of Demented Persons in the Community

Ruth L. Fischbach
*Department of Psychiatry, School of Medicine*
*Washington University, St. Louis, MO*

INTRODUCTION

Demographic trends, described as "the graying of America", require that we confront the social and economic impact posed by dementia. As the population surviving beyond their 60s and at risk for dementia increases, the burden placed on families and the cost to society at the micro and macro levels escalate. The cost for nursing home care alone is projected to increase to $75 billion in 1990 from $21 billion in 1981 (Terry and Katzman, 1983). At least 60% of nursing home residents are estimated to have SDAT (Kay and Bergmann, 1980; Brody et al., 1984), the most common form of dementia among the elderly.

At present, we cannot prevent dementia and know remarkably little about its natural history (Cooper and Bickel, 1984). A first, crucial step toward an improved understanding of this devastating disorder and toward improved care for demented patients is to enhance our ability to identify members of the community who show signs and symptoms of dementia. With this ability we would be able to develop the means to predict a) the onset of mild dementia, b) the speed with which mild dementia progresses to a severe form, c) the likelihood that dementia will be associated with personality distortion or depressed mood, d) the length of interval between onset of dementia and death, and e) the immediate cause of death.

There is great justification for identification of dementia in its earliest stages. These are well known: to identify individuals with dementias that are reversible or amenable to medical intervention (e.g., caused by medications, endocrine imbalances, tumors, normal pressure hydrocephalus)(see Larson et al., 1985 for an expanded discussion); to identify and subsequently enlist persons with dementia in prospective research protocols that would provide information about the natural history of the disease or the effectiveness of experimental drug therapies; to enlist persons with dementia into retrospective studies (e.g., case-control design) to provide information about exposures, heritability, or other risk factors; and finally to provide information essential for the formulation of health care policy to

ensure appropriate resources to care for the burgeoning number of people afflicted with dementia.

While the ability to identify individuals in the earliest stages of dementia would be particularly valuable to enable definitive diagnosis and early intervention, as Williamson et al. (1964) have shown, even in Scotland, where virtually everyone is enrolled with a general practitioner, most cases of dementia (and depression) in the community elderly were unknown to their doctors. More recently, O'Connor et al. (1988) and Waxman and Carner (1984) have demonstrated the failures of physicians or community nurses to recognize cognitive impairment (or depression) among their patients.

Despite recent investigations, the prevalence of Alzheimer's disease is not known with any precision and even less is known about the incidence (Henderson, 1986). In estimating rates of dementia, numerous variations make it difficult to obtain consistent information. Sources of variation include disparity in sample sizes, age ranges, means of identifying cases or defining severity, or using samples unrepresentative of the general population because they are treated cases (e.g., Kay et al., 1964; Pattie & Gilleard, 1978; Pfeffer et al., 1981; Christie, 1982) or because they are composed of volunteers (e.g., Gianturco and Busse, 1978; Hughes, et al., 1982). From Henderson's (1986) review of the field, followed more recently by the extensive integrative review by Jorm and his colleagues (1987), it is apparent that even if samples and ascertainment methods of existing studies had been standardized, the majority of these studies are now sufficiently dated that they do not reflect the increased survivorship given recent improvements in medical care. For example, of the 47 studies listed by Jorm et al., fewer than half were conducted in the last 10 years. In addition, few, 7 of 47, of these studies were conducted in the United States which raises questions as to how well the existing data represent the American experience.

In spite of the fact that the estimated number of cases of dementia has increased by a factor of 10 in this century (Mortimer and Hutton, 1985), studies of the incidence of dementia are limited, particularly from the United States, and published estimates vary widely (Mortimer, 1990; Henderson, 1986). The lack of standardized assessment criteria or the use of treated samples rather than probability samples make comparisons of rates difficult. The incidence of Alzheimer's disease (AD) is even more problematic to determine as few histopathologic diagnoses are made. Hence, rates for AD are usually extrapolated from the 50-60% of cases of dementia that on clinicopathological series (Tomlinson et al., 1970; Molsa et al., 1984) show the characteristic brain lesions (plaques and tangles) of AD.

Of the few published studies, Jarvik et al. (1980), in their follow-up of Kallman's 268 elderly twins, estimated incidence within six years in their sample (N=22) at 16%. Hagnell et al.

(1981), in a 25 year follow-up study, estimated an incidence rate for senile dementia in a rural Swedish sample of 0.7% by age 79 for men, 0.5% for women, and 1.9% by age 80+ for men and 2.5% for women. Mild forms of the disorders had correspondingly higher risks. They also reported a drop in incidence over the period of observation. Heyman et al. (1983) estimated the cumulative incidence at age 75 of dementia in siblings and parents of Alzheimer's disease probands at 14%. A 90-year lifetime incidence among relatives of Alzheimer's disease probands exceeded 50% in Breitner and Folstein's study (1984)!

Knowledge about dementia as it occurs in the general population comes principally from epidemiological studies (Henderson, 1990). Data are needed using large and representative samples surveyed with a standardized valid instrument. Incidence rather than prevalence studies are preferable as factors such as length of survival after onset may vary substantially across study sites. This might lead to striking differences in prevalence rates even when incidence rates are comparable (Mortimer, 1990). Data acquired in such studies could tell us whether, for example, the incidence rate plateaus at about age 75 or even declines at very advanced age as has been suggested by Hagnell et al. (1981) and Mortimer (1983). This suggestion seems plausible as the susceptible subgroup diminishes in size. A consistent sex ratio has not been reported across studies although more reports indicate higher incidence among females, particularly at advanced age (Mortimer, 1990).

Because of the great need to be able to identify persons in the community with dementia, this chapter is offered to suggest some instruments that are available that could be used for assessment and to describe a recently completed prospective study. Hopefully this chapter will provide some assistance and encouragement for researchers interested in furthering our knowledge in this area.

Why the Adult Lifestyle and Function Interview (ALFI) Study

We are aware of no previous systematic prospective assessment of a large community population entering the age of risk for dementia using standardized diagnostic criteria. The prospective design, which uses material collected from the individual himself or herself prior to the onset of dementia, has great strengths. Retrospective studies rely solely on reports by informants because dementia seriously compromises the affected individual's ability to report past disorders. As comparisons of family history and personal interviews with family members have shown (Leckman, 1982; Platt, 1980), reports by relatives often omit important subjective symptoms, probably because the affected person did not reveal them or there may be similar patterns of under- or over-reporting due to shared perceptions. On the other hand, as the disease progresses, the role of the informant becomes crucial due to the lack of insight, denial, or inability of affected persons to recall

or relate symptoms or other details about their condition. Therefore, in the ALFI study, an informant was interviewed to corroborate and elaborate on the information given by the respondent. Obtaining information independently from the proband and an informant has been called the best strategy for epidemiological studies of SDAT (Henderson, 1986).

A major strength of the ALFI study is its focus on a large representative sample of elderly persons carefully assessed over six years. St. Louis was one of five sites nationwide to participate in the Epidemiological Catchment Area (ECA) Project (Eaton and Kessler, 1985; Robins, et al., 1984; George et al., 1988). Starting in 1981 (Wave 1), a segment of the St. Louis area population, thirty percent of whom were then aged 60 years or older, were comprehensively assessed with respect to a lifetime history of psychiatric disorder and current dementia. Respondents were administered the Diagnostic Interview Schedule (DIS) (Robins et al., 1981) that includes a screening test for dementia, the Mini-Mental State Examination (MMSE)(Folstein et al., 1975). The MMSE covers a subset of the DSM-III criteria (American Psychiatric Association, 1980) for dementia and is widely used and recognized as a valid and reliable instrument (Anthony et al., 1982; Wolfson and Katzman, 1983; McKhann et al., 1984; Henderson, 1986) and the best brief objective cognitive test currently in use (Roth et al., 1986).

One year later (1982-1983) the sample was reinterviewed (Wave 2). Those elderly who passed the MMSE at this follow-up and thus were presumably <u>not</u> demented, were assessed for the third time in 1987 in a telephone interview using the Adult Lifestyle and Function Interview (ALFI).

There is general agreement that changes in personality, behavior, physical, and social function accompany the cognitive impairment of dementia (e.g., Roth, 1986). The ALFI study relied on a comprehensive instrument that systematically assessed these functions. The ALFI incorporates a variety of indicators including the Blessed Dementia Scale (Blessed et al., 1968); facets of the DIS that explore depression, alcohol abuse and dependence, tobacco use disorder, and delusions and hallucinations; The Index of Activities of Daily Living of Katz et al., 1970; Instrumental activities of daily living (adapted from Lawton and Brody, 1969); and questions designed to test several aspects of memory and cognition. Additional questions were designed to explore physical illnesses that might help distinguish multi-infarct dementia from SDAT or "probable Alzheimer's disease" according to NINCDS-ADRDA criteria (McKhann et al., 1984). Inclusion of several indices that had not previously been translated into specific questions that had clear scoring algorithms for responses, or which in previous studies were asked only of the informant, is a significant feature of this instrument.

Of particular interest was impairment in instrumental activities of daily living. Vitaliano et al. (1984) suggested these higher level activities may be the most sensitive to initial changes in AD. Fillenbaum and colleagues (1986), using cross-sectional ECA data from Duke, found these items to be the most important of the variables examined in explaining MMSE scores. With that in mind, we assessed how changes in function relate to MMSE changes and how they vary over time, comparing function over six years of surveillance.

The ALFI has been validated by referring 25% of the respondents with indicators of dementia to physicians and psychometricians of the Memory and Aging Project (MAP), the clinical core of Washington University's Alzheimer's Disease Research Center (ADRC).

While a major strength of the ALFI project was the collection of respondent self-reports as well as the reports of an informant, this chapter will focus on data from the respondent report.

## METHODS

### Design:

A total of 3,500 adult respondents, including 1,042 who were then aged 60 or older, were interviewed in the St. Louis Epidemiologic Catchment Area (ECA) Program beginning in 1981. They included both household (N=765) and institutional (N=277) residents. Blacks and nursing home residents were intentionally oversampled. Assessment was conducted using a specially designed instrument, the NIMH Diagnostic Interview Schedule (DIS) (Robins et al., 1981). Questions and probes included in this interview enable collection of data necessary to allow computer diagnosis of approximately 35 of the major psychiatric diagnoses covered by the American Psychiatric Association's Diagnostic and Statistical Manual Version III (DSM-III, 1980). The Mini-Mental State Exam (MMSE) (Folstein et al., 1975) is an integral part of the interview. Other domains are also assessed such as physical status, utilization of health care, use of medication, social functioning, family history, etc. Lay interviewers were trained in the administration of this instrument for approximately two weeks.

Using the 1980 census, ECA respondents were selected by area sampling in three noncontiguous catchment areas: the inner city, an inner suburban area adjacent to the city, and three largely rural counties. These three areas were chosen because together they were demographically similar to the State of Missouri and to the U.S. as a whole, demonstrating wide variation in degree of urbanization and social status. The inner-city area of St. Louis is the most disadvantaged in the state of Missouri; the suburb is composed of working class and middle class residents; and the third is a small town and rural area on the outskirts of the St. Louis

metropolitan area (Robins et al., 1984). Dwelling units in these areas were randomly selected in a multi-stage sampling process and, within each household, one respondent was randomly selected according to sampling tables designed by Kish (1965). The sample was designed to oversample blacks and residents of institutions and to select only one person from each household, regardless of household size. Because the sampling ratios and household membership are known, weighting allows reconstructing the adult populations of the sampled areas.

The design for the ECA Program called for two in-person interviews a year apart for all respondents plus an interim interview by telephone at six months for household residents in the community, i.e., those not institutionalized. For the initial survey, 80% of the designated households provided interviews with the randomly selected respondent. Follow-up assessment by telephone at six months and in-person at one year were completed with 86% of the survivors among those initially interviewed including 82% of the elderly sample.

ALFI sample

The sample for the ALFI study included both household-dwelling and institutionalized ECA respondents aged 65 years or older in 1986 when our study preparations began. They were selected from the original sample of elderly who demonstrated no or only minimal cognitive impairment at the time of their Wave 1 and Wave 2 in-person interviews conducted between 1981 and 1983. Ninety six percent of the sample at both assessments made 13 or fewer errors on the MMSE (out of a possible total of 30) while the remaining 4 percent made more than 13 errors at Wave 1 but 13 or fewer errors at Wave 2.

Attrition is always a problem in a longitudinal study, particularly in a elderly sample, and the ALFI study was no exception. From the original 1,042 ECA respondents aged 60 years or more, 633 were eligible (based on their MMSE scores) for the ALFI follow-up assessment. These were the respondents who were not cognitively impaired at Wave 2 and hence could be considered incident cases if subsequent impairment developed. We confirmed through an extensive search of death certificates that in the interim period between Wave 2 and ALFI, of the original 633, 139 (22%) of the respondents had died, a death rate of approximately 5% per year that is consistent with national mortality rates.

The "not interviewed" sub-sample (N=91, 18%) consisted of those who were not assessed for a variety of reasons. We were unable to locate 7% of the eligible sample (N=37). It is possible that some ECA respondents died outside of the state of Missouri as the search of Missouri death certificates did not reveal their names. It is also likely that many moved in with family or into a nursing home, leaving no forwarding phone number or address. A few were unable to be contacted because of the protectorship of

their guardians. The 18% also includes refusals, of which there were 39 (8%) and those unable to be interviewed because of poor physical or mental health (N = 15, 3%).

Of the 457 eligible elderly, i.e., alive and located, 403 were assessed, providing a 88.2% response rate. In addition to the respondents, an informant was interviewed who could both corroborate the respondent's answers and provide further information. Of the 403 respondents, 350 (86.8%) identified an informant who subsequently was interviewed.

Content of The ALFI Interview

Because this chapter was prepared with an aim to assist those who wish to learn more about the assessment of dementia in the community, the content of the ALFI will be explained in detail.

Although memory impairment is a hallmark of dementia, we believe other indicators which to date have not been definitively associated are present in the early insidious stages and these may be good predictors of the disorder. Therefore, we designed an instrument that was eclectic and comprehensive, reflecting a distillation both of variables suggested in the literature as indicators of dementia and indices used by prior investigators to determine prevalences of dementia. This highly structured interview operationalizes many well known assessment tools for dementia in a form that allows a trained lay interviewer to administer them by phone. It also repeats many items asked in the ECA to test consistency in reporting.

As a primary interest was the identification and assessment of dementia in a community sample, the ALFI contained a variety of relevant scales and measures including (1) criteria required by the NINCDS-ADRDA work group for "probable Alzheimer's Disease" (McKhann et al., 1984) and (2) criteria used for a suggestive clinical dementia rating (CDR) developed by Berg and colleagues (Berg, 1984; Hughes et al., 1982). The Mini-Mental Status Exam (MMSE) served as the central indicator of cognitive impairment. Sections also included from the Diagnostic Interview Schedule (DIS) assessed alcohol abuse/dependence and depression. The Blessed Dementia Scale checklist (Blessed et al., 1968), designed to be used only with informants, was operationalized so that respondents could now be assessed directly and their report compared to their informant's assessment. In addition, scales were developed to assess memory problems, ADL, IADL, hearing, vision, nutrition, smoking, medication patterns, health status, physical disorders, health utilization, and social functioning. The outline below presents topics or measures included in the ALFI with examples of specific items.

Outline of ALFI topics

1. Cognitive Impairment Assessment
   Mini-Mental State Exam

Recall of personal data: birthday, country of birth, telephone number, family history, occupation, major life events
Respondent's subjective assessment of memory problems: forgetting words, faces, events, use of appliances, getting lost
Interviewer's assessment: level of confusion, speech
Informant report
Memory and Aging Project clinical evaluation

2. Psychiatric Disorder
   DIS: depressive episode and symptoms, alcohol abuse/dependence, somatization and schizophrenic (psychotic) symptoms
3. Behavior/Personality Changes
   Blessed Dementia Scale: increased rigidity, apathy, coarsening of affect, purposeless hyperactivity
4. Physical Functioning
   DIS questions related to chronic disease (e.g., hypertension, stroke, diabetes, pulmonary, liver, kidney disorder)
   DIS questions related to utilization of health services, number of bed days, physician visits, hospital admissions
   DIS questions regarding self-rated health status
   Incontinence problems
   Recent falls, head trauma, neurological problems, Parkinson's disease, mobility
   NHANES assessment of vision and hearing
   Nutritional status: difficulties with eating, loss of teeth, appetite
   Rest and sleep patterns: number of hours sleep, out of bed, night wandering
   Activities of Daily Living: bathing, dressing, transfer, toileting, eating
   Instrumental activities of daily living: driving, shopping, handling finances
   Use of medications: psychoactive, prescription and non-prescribed drugs, recency (last month, last 2 days)
5. Social Functioning/Social Support
   Ability to do things for others, make visits, make phone calls, eating with others
   Have someone available if needed, use of formal and informal community services, recency of visits and phone calls
6. Miscellaneous: independence in living arrangements, decision making, financial status, satisfaction with current living conditions and personal life

To evaluate the possible onset of dementia, those parts of the MMSE which could be given by telephone were repeated. Of the 30 items in the full examination, nine items cannot be administered via the phone because they require that the respondent perform certain

activities that must be evaluated in person such as reading a sentence, writing a sentence, identifying an object presented by the interviewer, reproducing a geometric figure, or completing a complex task with a folded paper. By lowering the errors required for a positive test from 13 to 9, the analysis of the effect this had in the ECA was to create a few false positives (4.4%) but that virtually no true positives were missed. Almost all of the most predictive questions are able to be given by phone (Ashford et al., 1989).

Many items included in the Initial Subject Protocol (ISP) which is the basis for the Clinical Dementia Rating (CDR) (Hughes et al., 1982; Berg et al., 1982; Berg, 1984) developed by the Memory and Aging Project, were included in the ALFI. These items are based on a wide sample of cognitive performance in daily life and have the merit of carrying high face validity (Henderson and Huppert, 1984) and reliability (Burke et al., 1988). The CDR was developed as a staging instrument to classify dementing illness as absent, questionable, mild, moderate, and severe.

To obtain the respondent's subjective report of problems with memory and function, the respondent was asked about his or her perception of increased memory difficulties, difficulties in carrying out instrumental activities of living, paranoid ideas, and changes in the responsibilities he or she carries out. A series of demographic questions regarding personal data that were answered in the ECA project and repeated in the ALFI to check concordance suggested memory impairment and allowed assessment of the respondent's level of confusion.

The Dementia Scale of Blessed et al. (1968) is used as one of several measures to stage dementia in patients seen in the Memory and Aging Project (MAP). This behavioral checklist is the one scale that has been validated by neuropathologic findings (Hughes, et al., 1982). However, questions with which to determine its items prior to the ALFI study had never been specified and it had been used only with informants. The 22 items of the Blessed Dementia Scale that relate to performance of everyday activities, changes in habits, personality, interest, and drive were operationalized so that they were in a format that could be asked both of the respondent and of an informant directly and in a standard fashion.

The emergence or reemergence of two psychiatric disorders that are prevalent in elderly samples and the biological basis of which may be related to dementia, specifically alcohol dependence and/or abuse and depression, were ascertained via the DIS. Selected questions from the schizophrenia section of the DIS were also included. Drevets and Rubin (1988; Rubin, 1990) have observed that about 15% of mildly and perhaps 50% of severely demented subjects examined in the MAP exhibit psychotic symptoms such as delusions and hallucinations during the course of their illness.

The Index of Independence in Activities of Daily Living (Katz

et al., 1970; Katz, 1983) evaluates functional independence or dependence in the areas of bathing, dressing, transferring, continence, and feeding. This index, scored by a trained rater, has been found to have practical value as a longitudinal measure of change and a predictor of adaptive capacity because it uses well defined criteria and is sensitive to changes in self-care functions. We operationalized the items so they could be asked directly of both respondent and informant. Of importance was determining whether or not an activity could be performed independently, with some help, or whether total assistance was required.

In addition, sections from the NHANES I (DHHS, 1984) were adapted in order to assess sensory deficits in hearing and vision. Loss of hearing and presumably vision may accompany advancing cognitive impairment, leading to social isolation, development if paranoid ideation, and psychiatric reactions (Ruben and Kruger, 1983).

Administration of the Interview

The ALFI was designed as a telephone administered interview. The telephone rather than face-to-face administration was chosen for several reasons. It is less costly (Weeks et al., 1983) and research has shown that valid responses can be obtained, lengthy interviews completed, and sensitive issues successfully discussed (Dillman et al., 1976; O'Toole et al., 1986). Furthermore, Aneshensel et al. (1982a and 1982b) reported that telephone interview results were comparable to those of in-person interviews, including no differences in mental health reports as assessed by the Center for Epidemiologic Studies-Depression (CES-D) Scale. There is also evidence that the elderly prefer telephone interviews as they are often reluctant to invite strangers into their homes (Weinberg, 1983).

For 21 respondents who did not have access to a phone, who were physically unable to complete a phone interview or who had severe hearing impairment, an interviewer conducted the assessment at the respondent's residence. In-person interviews were conducted for all 49 nursing home residents. Hence, a total of 70 out of 403 (17.4%) of the interviews were person-to-person assessments.

Informant Interview

The respondent was asked for permission for us to speak with a relative or close friend about how he or she had been getting along and for that person's address and telephone number. Information provided by the respondent was corroborated and supplemented by this person. The informant, during a phone interview, was asked the same set of questions as the respondent as well as additional questions about certain behaviors and judgment that cannot be asked of the respondent. The operationalized Blessed Dementia Scale and Index of Activities of Daily Living were included. For obvious reasons, the only sections of the respondent version of the ALFI that were omitted were the MMSE and the DIS sections that required the respondent to report symptoms of depression, alcohol abuse/dependence, tobacco use

disorder, somatization, and schizophrenia. To substitute for these sections, informants were asked a global assessment of whether these disorders or symptoms appeared to be present. Informants were assessed by an interviewer who was unaware of the respondent report to avoid interviewer bias.

The Clinical Assessment

Respondents suspected of having an onset of dementia based on their responses on the ALFI were invited to be seen in person at the Memory and Aging Project (MAP). There they were given a battery of psychometric tests which have been shown by the MAP to correlate 0.98 with clinical assessment of senile dementia of the Alzheimer type (SDAT) in patients pre-screened for an absence of depression, alcoholism, and stroke. Their six-category clinical dementia rating has been shown reliable and useful for staging dementia in older subjects (Hughes et al., 1982). In addition, the full MMSE was administered for comparison with the ALFI MMSE, and the Hamilton Rating Scale (1967) for depression was administered to rule out the possible error of a positive diagnosis of dementia in someone who is only depressed. A physical examination was administered to check for neurologic or systemic disorders that might be causing dementia and to make the clinical diagnosis of SDAT. (Confirmation of the diagnosis of Alzheimer's disease is only by histopathologic evidence obtained from a biopsy or autopsy). The MAP assessment always included an interview with an informant who provided supplemental data including the items from the Blessed Dementia Scale. Testing was performed by the MAP professional staff. The assessment took approximately two hours. A small remuneration was offered to the respondents for their participation at the MAP.

Data Management and Analysis

Using data acquired from a probability sample that employed standardized criteria and clinical validation, data analysis has included estimating incidence rates, establishing an efficient set of functional predictors of dementia and their relationship to dementia, and an assessment of service utilization.

The general plan for data analysis encompassed four stages: (1) data editing, (2) data reduction, (3) effect estimation, and (4) hypothesis testing, stratification, and multivariate analysis. The wealth of data provided by the serial assessments and the comprehensive interview schedule make many analyses possible and therefore the data presented in this chapter are merely the beginning of a series of analyses to be made.

After the data were edited (e.g., checked for consistency, open-ended responses coded), they were summarized using descriptive statistics. This entailed merging the three data sets from the ECA project (Wave 1, telephone, Wave 2) plus the data from the ALFI including respondent and informant reports and data for the 25 subjects evaluated at the MAP. Contingency tables were generated for the distributions of the primary outcome variables (dementia, levels

of function, use of services) according to age, gender, and race. Scales, based on the items listed in the outline above, were developed to measure change in cognitive, psychiatric, behavioral, physical, and social function. Cumulative incidence rates for dementia that are age-, sex-, and race-specific were estimated. Also computed were prevalences for major depressive episode, alcohol abuse and/or dependence, and tobacco use disorder using both the ECA and ALFI data sets. Multivariable analysis has suggested models to predict onset of subsequent dementia assessed at ALFI from ECA data.

Dependent variables

Four variables were constructed to reflect the presence or level of cognitive impairment or dementia among ALFI respondents. The first variable was based on the sum of errors made on the MMSE. Using the scoring convention employed in the ECA assessment, total errors included "don't know" and refused responses. The assumption made was that if the respondent had been able to provide the correct answer, he/she would have done so. The best score of the serial 7s or spelling WORLD backwards was used. The range of values for the ALFI MMSE was from 0 to 21, with scores equal to or greater than 9 considered "failing" and severe cognitive impairment being present. This "cut point" is equivalent to the 13 errors used by the ECA to indicate severe cognitive impairment. The dichotomous variable used in contingency tables to indicate "passing" or "failing" is referred to as "Failed MMSE."

The second variable, referred to as "impairment status," is a severity measure of cognitive impairment which also uses ECA scoring methods. The ALFI MMSE scores were categorized into three levels according to total MMSE errors: 0-3 errors = well; 4-8 errors = mild/subclinical; 9+ errors = severe cognitive impairment/dementia.

Because of the reported association of the MMSE to race and educational level (Anthony et al., 1982), a third and more inclusive measure which includes items unrelated to ethnicity and educational attainment was constructed based on an algorithm that included:

1. the MMSE score
2. the failure to recall a variety of items that "anyone" ought to know such as "are you married?", "have you had a visitor in the last 2 weeks?", and "what are your children's names", "who lives with you?"
3. the interviewer's assessment of possible dementia
4. the respondent's self-report of memory problems such as inability to: find the right word, recall what had just been told, remember appointments, get to places, find way in the neighborhood, learn how to use gadget
5. the informant interview that suggested dementia

In greater detail, the construction of this assessment variable, which appears to be an excellent indicator of mild dementia, is as follows. Respondents were classified as (1) "definite" case (if MMSE score 9+ or failed to remember 6+ personal recall items or interviewer coded "appears severely demented"); (2) questionable case

(if MMSE 4-8 errors or interviewer coded "appears mildly demented" and failed to remember 3-5 of the recall items or reports 6+ memory problems or informant interview indicates mild or severe dementia); or (3) "well". Those who met criteria for definite or questionable case were eligible to be invited in, i.e., eligible for referral, to the MAP to receive a clinical assessment (described above), hence this variable is referred to in the tables as "MAPR."

The fourth constructed variable reflected the change in MMSE scores over the six-year surveillance period between Wave 1 and the ALFI. The MMSE scores at each wave were first categorized into three levels as described above (0-3 errors well; 4-8 errors = mild/subclinical; 9+ errors = severe cognitive impairment/dementia). Respondents were assigned to a mutually exclusive category according to whether their scores across the waves remained well, improved, became worse, or were consistently poor. This variable is referred to as "change in cognitive impairment over 6 years."

Impairment in behavior and personality using the Blessed Dementia Scale, physical function using the measures of ADL and IADL, mobility, and days of limited activity or confinement to bed, and social function using variables from the ALFI were also categorized. Activity was dichotomized as either completely independent or dependent when used in analyses requiring nominal level variables and as a sum of three levels (scale) when used in analyses requiring continuous variables. Chi square tests or Fishers exact tests were used in the contingency tables and Pearson r correlation coefficients were used in the correlation matrices.

## RESULTS

### Evaluation of the telephone as a means to collect data

Several unique features of the ALFI study should be noted. First, unlike the ECA study, interviews were conducted primarily by telephone. Some may be skeptical about using a telephone interview to assess an elderly sample, particularly when the goal is to identity dementia. The ALFI data support the efficacy and validity of this data collection method, however. The response rate was good, 86%, and the proportion of those with no or very few errors on the MMSE was consistent with ECA scores obtained in the face-to-face interviews. Respondents had positive comments about the interview, many reporting they enjoyed the interview despite the fact that the mean number of minutes to complete the assessment was 60. But most importantly, a clinical assessment of respondents was used to validate data gathered by the phone interview that suggested dementia. Thirty six percent (N=25) of those considered demented (N=70) were seen by clinicians from the Memory and Aging Project, the clinical component of the ADRC. Replication of the MMSE indicated good concordance in the scores. In addition, the ALFI study included nursing home residents who were interviewed in person providing an opportunity to compare face-to-face interviews with telephone

interviews. There were no differences in refusal rate or in validation of the MMSE.

Description of the ALFI Sample

Data on Table 1, represent the demographic characteristics of the group eligible for the ALFI follow-up as they were in 1981. The interview status indicates the 1986-87 outcome of our effort to locate and interview this group, i.e., whether the ECA respondents were interviewed, were confirmed to be deceased, or were never located. Computing the row percentages, as can be noted, whereas females were somewhat over-represented in the interviewed group, males in the deceased group were disproportionately represented relative to their proportion in the other two groups. The mean ages of the status groups ranged from 70.0 (SD = 7.5) for the interviewed group, 70.5 (SD= 7.6) for the no contact group and 75.1 (SD = 8.4) for the deceased group ($p<0.0001$). The majority (55%) of the original 42 ECA oldest-old respondents were in the deceased group as would be expected. There was more equitable distribution among the sub-samples for race, education, and marital status. In data not shown, the distribution of elderly in the ALFI follow-up study in the oldest-old category, i.e., those 85 years and older, rose in 6 years to 15% (N=59) from 4% (N=16), indicating that even with their higher mortality, this portion of the population is seeing a dramatic rise in numbers relative to other age groups.

Table 2 provides the description of the ALFI respondents at the six-year follow-up (1987) comparing the household (community dwelling) and institutional samples. Relative to the household sample, the institutional group has more males, is older, has notably few blacks, is somewhat more educated, is predominantly composed of widows or those who had never married, and has slightly fewer professional workers.

Distribution of dementia and cognitive impairment

Presented on Table 3 is the distribution of dementia in the household sample, using the comprehensive variable MAPR, and the distribution of cognitive impairment, using the cut point of 9 or more errors on the MMSE (the equivalent of 13 errors used during the ECA assessment). As shown, whereas there is no gender difference, blacks are disproportionately represented along with those with fewer years of education in the demented and cognitively impaired categories. These results are particularly intriguing as all ALFI respondents had received "passing" scores on the MMSE in Wave 2 and 96% in Wave 1. Hence, it is problematic to attribute these differences to a racial or educational bias of the MMSE. Dementia/Impairment appears to increase in direct proportion with age with the largest increase occurring at or beyond age 85. Data pertaining to the nursing home sample is not shown but demonstrate the same distributions.

Incidence

The five-year cumulative incidence rate for cognitive impairment

was determined using the MMSE score. Incidence is calculated as the number of persons developing the disorder per unit time divided by the total number at risk, i.e., 49/403 x 5. The estimated cumulative incidence rate is 0.122 or 0.024 per year which suggests 2,400 new cases of cognitive impairment per 100,000 population may be expected. In estimating the rate using the MAPR variable, (70/403 x 5), the cumulative incidence rate is 0.176 or 0.035 per year. The prediction is that 3,520 persons per year per 100,000 population would develop dementia. One could extrapolate, using the suggested percentages of 50-60% of dementia cases being SDAT, and from the ALFI data project that between 1,700-2,000 persons per year per 100,000 may have dementia of the Alzheimer's type.

The development of dementia is strongly related to age. Table 4 presents our four measures comparing younger (65-84 years) and older (85+ years) age groups in the household sample. The older group is more than twice as likely to be both afflicted and to experience a worsening of cognitive status than the younger group. In data not shown, the association of dementia with age was strong even when controlling for race. Of interest was the particularly striking proportion of the black subsample whose impairment worsened relative to the subsample of whites. Also in data not shown, the rate of dementia, measured by MAPR, ascends steadily in whites with the steepest increase after age 85 while the rate in blacks starts off higher (28 % v. 7 % at age 65) and fluctuates with the steepest increase at age 80 followed by a dip to age 90+ years. This fluctuation may be due to the small black sample in the oldest age group. In the white sample, the rate of dementia does not plateau even at the age of 90, which had been suggested by other research (e.g., Hagnell et al., 1981; Mortimer, 1983). The association of dementia and age holds when controlling for education, as in both age groups the proportion of dementia is lower in those with nine or more years of education. It is interesting to speculate about the protective effect of education.

The literature has provided equivocal data regarding the association of dementia with gender (Mortimer, 1990). Figure 1 presents the ALFI data which indicate no statistically significant association between dementia and gender but demonstrate how the rate of dementia increases with age in both genders. The rate of increase is more consistent in males as there appears to be a plateau effect among the females between ages 70 - 80. While it appears that the increase in the rate of dementia in males precedes the increase in females, these differences are not statistically significant.

Nursing home residents

Because we surveyed both household and institutional residents over six years, we have some indication of the movement of respondents into and out of nursing homes. Data indicate that the vast majority, 87%, remained in the community as household dwellers while 9% were in nursing homes at both assessments. Only 14 persons (3%) entered an institution during this six year period while 2

people (< 1 %) left and went into a household.

The distribution of cognitive impairment status among the household and nursing home residents is displayed in figure 2. Whereas 66% of the household dwellers are considered "well," only 30% of the nursing home residents are in this category. At the other end of the spectrum, only 11% of the household residents fell into the "severely impaired" category compared to 30% of the nursing home residents.

The four measures of dementia/cognitive impairment were examined according to place of residence (data not shown). As might be expected, those living in households had lower percentages of dementia and impairment. Those who moved into an institutional setting had the highest failure rate on the MMSE and also showed a change for the worse in their cognitive ability.

Comorbidity

ALFI data provided information related to comorbidity with dementia. Alcohol and depression symptoms were examined. Very few (N=7) reported any symptoms related to alcohol abuse or dependence at the ALFI assessment although larger numbers reported symptoms at the earlier ECA assessments. Depressive symptoms were reported more frequently than alcohol symptoms and their relationship with dementia is presented in Table 5. While the suggestion that over one half of the non-demented sample (123/223) reports no depressive symptoms as compared to the impaired groups, what is particularly striking is the proportion of respondents categorized as severely demented who report three or more symptoms. This finding emphasizes the overlay of depression that frequently accompanies dementia.

Summary

The ability to reliably and efficiently identify persons in the community who are demented is needed. To promote our ability to make these assessments, the construction of a new comprehensive instrument that can be administered by a lay interviewer via the telephone was described. Also described was a longitudinal study based on a large community sample. Many of the methodologic problems invariably associated with a prospective study of an elderly population have been discussed. Data from this study should allow the formulation and testing of important hypotheses related to the onset and progression of dementia in a community sample. What is clear is that there is a large proportion of community residents who are currently demented. In view of demographic trends, the size of this group will increase dramatically. As a society we will be challenged to provide appropriate care for these individuals and support for their families. And we will rely on research efforts to provide information that may ultimately identify the cause(s) and offer the long awaited cure for this terrible disorder.

TABLE 1

INTERVIEW STATUS OF THOSE ELIGIBLE FOR A SIX-YEAR FOLLOW-UP
WAVE 1 DATA: (1981)
N = 633

| | INTERVIEWED (N =403) | | DECEASED (N= 139) | | NO CONTACT (N = 91) | |
|---|---|---|---|---|---|---|
| | f | % | f | % | f | % |
| GENDER | | | | | | |
| Male | (133) | 57 | (67) | 29 | (31) | 13 |
| Female | (270) | 67 | (72) | 18 | (60) | 15 |
| AGE | | | | | | |
| 60 - 74 | (299) | 70 | (70) | 16 | (60) | 14 |
| 75 - 84 | ( 88) | 54 | (46) | 28 | (28) | 17 |
| 85+ | ( 16) | 38 | (23) | 55 | ( 3) | 7 |
| RACE | | | | | | |
| White/ Other | (282) | 63 | (98) | 22 | (69) | 15 |
| Black | (121) | 66 | (41) | 22 | (22) | 12 |
| EDUCATION | | | | | | |
| 8 or less | (202) | 60 | (82) | 24 | (53) | 16 |
| 9+ | (201) | 68 | (57) | 19 | (38) | 13 |
| MARITAL STATUS | | | | | | |
| Married | (140) | 65 | (40) | 19 | (35) | 16 |
| Wid/Div Sep | (221) | 62 | (87) | 25 | (48) | 14 |
| Never Married | ( 42) | 68 | (12) | 19 | ( 8) | 13 |

TABLE 2

DEMOGRAPHIC DESCRIPTION OF THE ALFI SAMPLE IN 1987

| | Location at ALFI Follow-up | | | | | |
|---|---|---|---|---|---|---|
| | Household (N = 354) | | Institution (N =49) | | Total (N = 403) | |
| | (f) | % | (f) | % | (f) | % |
| Gender | | | | | | |
| Male | (113) | 32 | (20) | 41 | (133) | 33 |
| Female | (241) | 68 | (29) | 59 | (270) | 67 |
| Age | | | | | | |
| 65-74 | (175) | 49 | (15) | 31 | (190) | 47 |
| 75-84 | (135) | 38 | (19) | 39 | (154) | 38 |
| 85+ | (44) | 12 | (15) | 31 | (59) | 15 |
| Race | | | | | | |
| Black | (113) | 32 | (8) | 16 | (121) | 30 |
| White | (241) | 68 | (41) | 84 | (282) | 70 |
| Education | | | | | | |
| 0-8 years | (179) | 51 | (23) | 47 | (202) | 50 |
| 9+ years | (175) | 49 | (26) | 53 | (201) | 50 |
| Marital Status | | | | | | |
| Married | (119) | 34 | (4) | 8 | (123) | 31 |
| Wid./Div./Sep. | (206) | 58 | (31) | 63 | (237) | 59 |
| Never Married | (29) | 8 | (14) | 29 | (43) | 11 |
| Make-up of Household | | | | | | |
| Lives alone | (180) | 51 | (0) | 0 | (181) | 45 |
| With Others | (174) | 49 | (49) | 100 | (222) | 55 |
| Socioeconomic Status +# | | | | | | |
| Professional | (125) | 36 | (16) | 33 | (141) | 35 |
| Unskilled | (227) | 64 | (33) | 67 | (260) | 65 |
| Sample | | | | | | |
| Household | | | | | (354) | 88 |
| Institution | | | | | (49) | 12 |

+ missing from sample = 2
# Hollingshead Scale

TABLE 3

DISTRIBUTION OF DEMENTIA AND COGNITIVE IMPAIRMENT
IN THE HOUSEHOLD SAMPLE
(N=354)

| | Demented (Eligible for MAPR) N=49 | | Cognitively impaired (Failed MMSE) N=35 | |
|---|---|---|---|---|
| | f | % | f | % |
| Gender | | | | |
| Male | 113 | 13 | 110 | 10 |
| Female | 241 | 14 | 225 | 11 |
| Age ** | | | | |
| 65-74 | 175 | 10 | 171 | 8 |
| 75-84 | 135 | 13 | 125 | 10 |
| 85+ | 44 | 30 | 39 | 23 |
| Race *** | | | | |
| Black | 113 | 29 | 103 | 26 |
| White | 241 | 7 | 232 | 3 |
| Education *** | | | | |
| 0-8 yrs | 179 | 21 | 165 | 19 |
| 9+ yrs | 175 | 7 | 170 | 2 |
| Marital Status | | | | |
| Married | 119 | 9 | 117 | 8 |
| Wid./Div. Sep. | 206 | 17 | 189 | 12 |
| Never Married | 29 | 10 | 29 | 10 |
| Make-up of Household | | | | |
| Lives alone | 180 | 14 | 167 | 10 |
| With others | 174 | 13 | 168 | 11 |
| Socioeconomic Status ** | | | | |
| Profess. | 125 | 9 | 121 | 3 |
| Unskilled | 227 | 16 | 213 | 14 |
| Sample | | | | |
| Household | 354 | 14 | 335 | 10 |

Note: 19 respondents were unable to complete the MMSE.

* p <0.05
** p <0.01
*** p <0.0001

TABLE 4

FOUR MEASURES OF COGNITIVE IMPAIRMENT/DEMENTIA IN THE HOUSEHOLD SAMPLE ACCORDING TO YOUNGER (N = 310) AND OLDER (N = 44) AGE GROUPS

| | Younger (65-84 years) f | % | Older (85+ years) f | % | *p* |
|---|---|---|---|---|---|
| Failed MMSE | | | | | |
| NO | (272) | 91 | (31) | 76 | .005 |
| YES | (26) | 9 | (10) | 24 | |
| Eligible for MAPR | | | | | |
| NO | (274) | 88 | (31) | 70 | .004 |
| YES | (36) | 12 | (13) | 30 | |
| Impairment Status | | | | | |
| Well | (205) | 69 | (18) | 44 | .001 |
| Mild | (65) | 22 | (13) | 32 | |
| Severe | (26) | 9 | (10) | 24 | |
| Change in Cognitive Impairment over 6 years | | | | | |
| Stayed Well | (181) | 67 | (16) | 46 | .01 |
| Improved | (8) | 3 | (0) | 0 | |
| Got Worse | (58) | 21 | (16) | 46 | |
| Stayed Impaired | (23) | 9 | (3) | 9 | |

Note: There are 17 missing who could not complete the MMSE

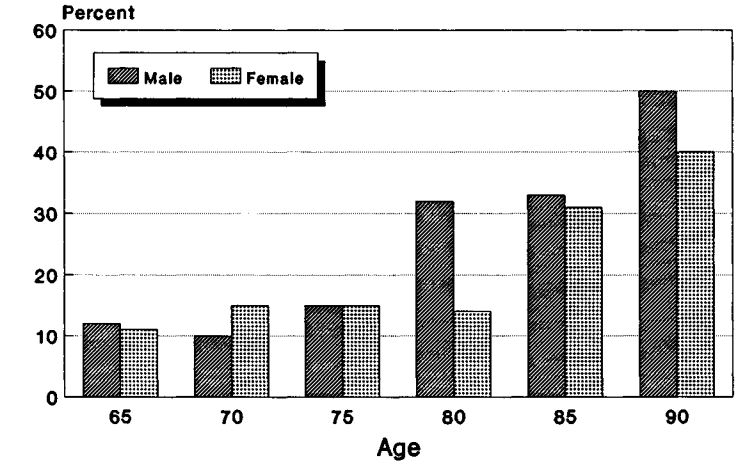
TABLE 5

ASSOCIATION OF COGNITIVE IMPAIRMENT AND SYMPTOMS OF DEPRESSION IN THE HOUSEHOLD SAMPLE ( N = 354)

| Number of Depressive Symptoms | Well f | % | Mild f | % | Severe f | % |
|---|---|---|---|---|---|---|
| None | (123) | 55 | (32) | 36 | (11) | 39 |
| 1-2 | ( 63) | 28 | (35) | 39 | ( 4) | 14 |
| 3+ | ( 37) | 17 | (22) | 25 | (13) | 46 |

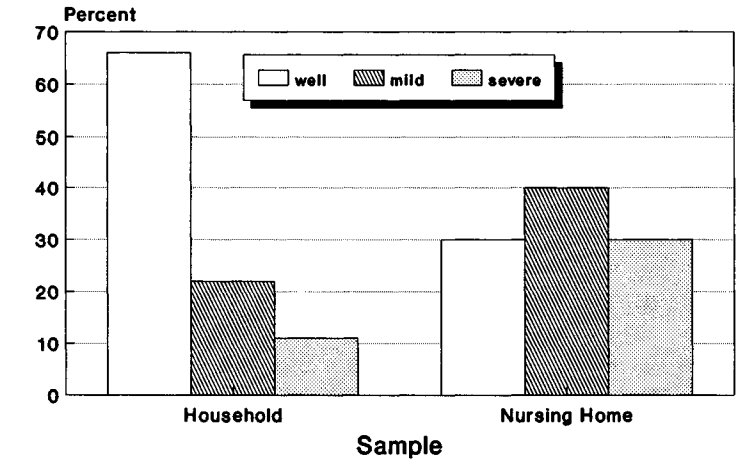
$p < 0.0001$

FIGURE 1




Dementia According to MAPR Status

FIGURE 2




P <0.001

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ACKNOWLEDGEMENTS

I would like to acknowledge the collaboration with Lee Robins, Ph.D. and members of the Memory and Aging Project of Washington University. This research was supported in part by grants from the National Institute on Aging (AG03991) and the Missouri Alzheimer's Disease Association.

After June 1, 1990, address requests for further information to Ruth L. Fischbach, Ph.D., Department of Social Medicine, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115

# Part 2

# Neuropsychology

# Neuropsychological Characteristics of Early Alzheimer Disease

Maura Mitrushina
*University of California, Los Angeles, CA*
Paula Altman Fuld
*Clinical and Consulting Associates, Encino, CA* and
*The Albert Einstein College of Medicine, Bronx, NY*

In this chapter, we focus on the possibility of "pre-clinical" detection of Alzheimer-type Dementia (AD). Currently, the criteria for the clinical diagnosis of AD require observable deficits in cognitive functioning (McKhann, Drachman, Folstein, Katzman, Price, & Stadlan, 1984). Recent research, however, indicates that it may soon be possible to predict AD in normal-functioning elderly (Fuld, Dickinson, Crystal, & Aronson, 1987; Katzman, Terry, DeTeresa, Brown, Davies, Fuld, Renbing, & Peck, 1988). To make a clinical diagnosis of Alzheimer-type dementia under current criteria is to hypothesize a specific form of biological degeneration underlying cognitive difficulties which are severe enough to interfere with every-day social and occupational functioning and of which the patient (or significant other) complains. To speak of "pre-clinical diagnosis" is to make a radical change in the current definition of dementia, in that "pre-clinical" implies "not yet any clinically significant complaint" of significant cognitive change which would interfere with social or occupational functioning.

Evaluating individuals who do not yet have any clinically significant complaint of cognitive change is hazardous for a number of reasons.

1. Biological changes may occur before changes in cognitive functions. If this is the case, one would have to develop accurate measures to distinguish early cognitive changes from premorbid or unimpaired functioning (which may include changes normally associated with aging). The first section of this chapter reviews the literature related to this problem. This section also shows that at the present time we are unable to make this discrimination due to:
   a. Diagnostic criteria which are meant for a later stage of dementia;
   b. The use of measurement instruments which are sensitive only to gross cognitive impairment;
   c. Substantial flaws in research methodology.

We argue that only longitudinal comparison of individuals' functioning against baseline data can permit accurate documentation of cognitive changes in still relatively preserved individuals. This premise is supported by review of findings obtained in the few longitudinal studies available to-date.

2. Frequently, in the clinic, we have to make diagnostic decisions based on the data gathered at one point in time. This can be aided by assessment of cognitive deficit patterns with focus on the domains which might be particularly vulnerable to AD etiology. In the early stages of AD the most reliable impairment is in the domain of memory. Memory impairment, however, occurs in most other conditions with which dementia could be confused, such as depression, delirium, and other causes of brain damage such as stroke.

BIOLOGICAL CHANGES APPEAR TO OCCUR BEFORE THE FIRST MANIFESTATIONS OF COGNITIVE IMPAIRMENT

There is a remarkable similarity between early symptoms of AD and changes associated with normal aging and dementias of different etiology. In deceased individuals, however, it is possible, in most cases, to differentiate AD from normal aging by identifying a number of neuropathological and chemical abnormalities in AD patients. Postmortem studies show a sharply reduced amount of choline acetyltransferase and somatostatin in the brains of Alzheimer patients (Katzman, Terry, DeTeresa, Brown, Davies, Fuld, Renbing, & Peck, 1988). There is also a high count of cortical senile plaques, neurofibrillary tangles, and granulo-vacuolar degeneration (Tomlinson, 1982; Kopelman, 1986a). Davidson (1987) speculated that neurons containing tangles were unable to receive vitally important nutrients, and that these neurons therefore degenerated. Arendt and Bigl (1987) further developed this assumption and suggested that when the extent of neuronal degeneration exceeds a threshold, the system decompensates and clinical symptoms become apparent. On the gross level, many studies show CT findings of cerebral atrophy, particularly in the frontal lobes and hippocampus (Terry, Peck, DeTeresa, Schecter, & Horoupian, 1981; Terry & Katzman, 1983). Thus, there is general agreement regarding the association of specific biological changes with AD.

With respect to the problem of early or pre-clinical diagnosis of AD, the strength and uniqueness of this association is crucial. Recent studies suggest that the presence of the biological substrate of AD, such as plaques and tangles, may or may not not be associated with AD-like cognitive deterioration. In a study reported by Fuld, Dickson, Crystal & Aronson (1987), senile plaques were significantly correlated with memory deterioration even in elderly persons without dementia. Correlation between the number of primitive plaques (which lack well-defined, compact, amyloid cores) with recall on the Fuld Object-Memory Evaluation was significant at .002 level ($r=-.81$). The authors suggested that these individuals were in the early, or "pre-clinical" stage of dementia, but it is also possible that they would not have gone on to manifest dementia had they not died.

Katzman et al., (1988) reported a postmortem study of 137 residents of a nursing facility whose cognitive status had been evaluated during life. Ten subjects from this sample showed the pathological features of mild Alzheimer's disease, with many neocortical plaques, although their cognitive performance was in the upper quintile of the nursing home residents, and as good as or better than the performance of the control subjects without brain pathology. Interestingly, these subjects were found to have higher brain weights and greater number of neurons than age-matched

control subjects. The authors speculated that these subjects may have had incipient AD but escaped loss of large neurons, or started with larger brains and a higher number of large neurons and thus have had a greater reserve.

Neuropathological changes in nondemented patients in their sixth and seventh decade were also reported by Ulrich (1985), and similar histologic changes in minimally cognitively impaired 85-year-old patient were described by Morris and Fulling (1988). Crystal, Dickson, Fuld, Masur, Scott, Mehler, Masdeu, Kawas, Aronson, & Wolfson (1988) reported in a study of 28 longitudinally evaluated elderly subjects that the number of senile plaques did not distinguish well between demented and nondemented subjects, although every subject with numerous cortical neurofibrillary tangles was demented. The authors hypothesized that nondemented subjects with postmortem neuropathology may have had "preclinical" AD, or numerous cortical plaques might occur in some elderly subjects who would never develop clinical dementia. Terry and Katzman (1983) reported the presence of neuritic plaques in the neocortex of up to 80% of all cases by the age of 90, and neurofibrillary tangles in the hippocampi of about 90% of cases. However, AD cases could be differentiated from normal aging by substantially higher numbers of plaques and tangles, and by the presence of neurofibrillary tangles in the neocortex. These observations are consistent with the results of longitudinal examination by PET scan of the early neuropsychological and cerebral metabolic changes in AD, reported by Grady and coworkers (Grady, Haxby, Sundram, Berg, & Rapoport, 1985; Haxby, Geady, Friedland, & Rapoport, 1987; Grady, Haxby, Horwitz, Sundaram, Berg, Schapiro, Friedland, & Rapoport, 1988). They showed that neocortical metabolic abnormalities usually precede the first manifestations of cognitive impairment by 8 to 16 months.

These studies suggest that the development of neuropathologic changes in the brain antedates observable clinical and neuropsychological manifestations of cognitive deficits. Hypothetically, people with a small number of senile plaques at the onset of disease might still remain asymptomatic or might show only mild memory difficulties. without other types of dysfunction to support a diagnosis of dementia. In addition, some people might have greater neurological and cognitive reserve, i.e. more advanced neuronal organization and higher premorbid level of cognitive functioning, which would delay their approaching the threshold for decompensation. Thus, the question of early diagnosis of AD remains problematic.

## SOURCES OF LIMITATIONS IN POTENTIAL ACCURACY OF EARLY DIAGNOSIS

NINCDS-ADRDA clinical criteria for probable AD (McKhann, Drachman, Folstein, Katzman, Price, & Stadlan, 1984) require the following behavioral manifestations of disorder: deficient performance on Mini-Mental Test or Blessed Dementia Scale and on neuropsychological tests; deficits in two or more areas of cognition; progressive worsening of memory and other cognitive functions (supported by deterioration of language, motor skills, and perception).

Similarly, research diagnostic criteria for senile dementia of Alzheimer type (SDAT) (Berg, Hughes, Coben, Danziger, Martin, Knesevich, 1982) include sustained deterioration of memory and impairment in at least three of the following five cognitive

domains: 1) orientation; 2) judgment and problem solving, 3) function in community affairs; 4) function in home and hobbies; 5) function in personal care. All of these symptoms should have gradual onset and duration for at least six months.

As follows from these clinical and research criteria, diagnosis of probable SDAT is warranted only when deterioration of memory and other cognitive capacities is advanced enough to cause observable behavioral deficits. Unfortunately, changes which are observable on CT or EEG studies do not occur early enough in AD for these to be useful in early diagnosis (Morris & Kopelman, 1986).

Another problem arises from inappropriate measurement instruments used in early identification of AD. It has been suggested that level of cognitive deterioration in dementia progresses in parallel with advancement in stage of neuroanatomical degeneration (Jolles & Hijman, 1983; Jolles, 1985). However, the focus and extent of anatomical involvement and of associated behavioral manifestations, differ with the progression of the disease. Tests that are sensitive to the cognitive impairment associated with more advanced stages of the disease, do not pick up subtle changes at the early stages.

The first subtle signs experienced by AD patients at the onset of the disease are related to memory difficulties (Sim & Sussman, 1962; Coblentz, Mattis, Zingesser, Kassoff, Wisniewski, & Katzman, 1973; Albert, 1981; Fuld, 1984a; Larrabee, Largen, & Levin, 1985; Morris & Kopelman, 1986; Grady et al. 1988). However, the most frequently used diagnostic procedures are mental status tests and brief dementia rating scales which provide relatively gross measures of the presence of a significant degree of cognitive or memory impairment (Zarit, Miller, & Kahn, 1978; Zarit, Eiler, & Hassinger, 1985; Jorm, 1986). These global measures of cognitive impairment are based on clinical experience, rather than on theoretical analysis of early cognitive changes in AD. Some do not assess memory for new information at all, except by addressing orientation to time and place. Further, validation of these scales was performed on institutionalized patients with rather advanced disease (Jorm, 1986). Therefore, it is not surprising that these scales have high discriminative validity assessing more advanced stages of the disease, and are insensitive to early cognitive changes associated with AD. Fillenbaum (1980) reported low accuracy in detection of AD victims in a community sample with the Mental Status Questionnaire (Kahn, Goldfarb, Pollack, & Peck, 1960) and with the Short Portable Mental Status Questionnaire (Pfeiffer, 1975). The hit rate for AD cases using these two instruments was only 55%. The hit rate for the Mini-Mental State (Folstein, Folstein, & McHugh, 1975) in assessing patients from a general hospital was higher: 87% of AD victims were correctly identified (Jorm, 1986). However, a majority of the patients participating in this study had already developed clinically observable cognitive deficits. Thus, mental status questionnaires have high value in detection of probable AD only when cognitive changes meet current clinical criteria. The task of early identification of AD can be accomplished only through assessment of subtle changes in cognitive capacities using more sensitive instruments than Mental Status Scales.

Assessment instruments that have proved to be more appropriate for early identification of cognitive changes are based on

multidimentional evaluation of cognitive functioning or on experimental paradigms for investigation of learning and memory. The Dementia Rating Scale (Mattis, 1976) evaluates a number of cognitive domains, such as attention and memory, initiation, perseveration, conceptualization, and construction, on the level of difficulty appropriate for early stages of disease. The Geriatric Mental Status Interview (Gurland, Copeland, Sharpe, & Kelleher, 1976) taps cognitive functioning, affective state, behavioral symptoms, and somatic concerns. The Multidimensional Assessment for Dementia Scales (Drachman, Fleming, & Glasser, 1982) evaluate seven functional parameters, which cover neurological, psychometric, and observational aspects. Another comprehensive battery, The New York University Memory Test (Randt, Brown & Osborne, 1980), taps seven modules of memory functioning. While these batteries are sensitive to indices of early cognitive deterioration, there has been insufficient study of their value in differentiating between AD and other sources of cognitive dysfunction, particularly age- and affect-related cognitive changes.

Several studies (Kahn and Miller, 1978; Anthony, Le Resche, Niaz, Von Korff, & Folstein, 1982) showed that mild cognitive disturbances due to early AD process are difficult to differentiate from problems caused by depression and other affective states, inadequate education, low premorbid level of intellectual functioning and motivation. Due to a remarkable similarity of these processes, attempt to make diagnostic decisions in such cases inevitably results in an overidentification of AD. Kahn and Miller (1978) believe that overidentification is far more harmful than underidentification, since it leads to the acceptance of a spurious organic diagnosis and precludes timely intervention. Overdiagnosis may even cause iatrogenic cognitive dysfunction due to reactive anxiety and depression.

Problems with Research Methods

Problems with early identification of AD are further exacerbated by substantial flaws in the research methodology used in studies of the cognitive sequelae of AD.

1. The majority of studies focus on differences in mean performance levels between different clinical and control groups. To show that a test differentiates between groups of diseased vs. non-diseased individuals does not indicate that the test would be useful for differentiation in individual cases. The extent of individual deviations from the mean would have to be considered, because in an individual case (a clinical situation), the extent of impairment relative to a group mean is pitted against a relatively great probability of error. In a study comparing groups, a very small difference between the groups may be statistically significant; in an individual patient, an impairment of that size can occur purely by chance. Also, a much larger degree of impairment would have to be observed for the examiner to be fairly sure that a true change in ability has occurred at all. To some extent, this potential source of error can be handled by repeated studies of the same individual.

2. Identification of early stages of dementia in each individual case is dependent on the baseline level of abilities. People, who have a high premorbid level of intellectual functioning may have greater "reserve", are more resistant to decline, and are

more likely to develop compensatory strategies masking incipient cognitive deficits. Early AD in these cases may be detectable only longitudinally, by measuring decline between consecutive testing probes, rather than by comparison of individual performance to the expected standards. This problem may, to some extent, be handled by making available normative data for groups of elderly individuals at various levels of ability.

3. Neuropsychological measures used for the identification of early AD in elderly people frequently do not have appropriate norms for this age group. Fortunately, long-needed age-appropriate normative data for many commonly used tests will be available soon (D'Elia and Boone,in press).

4. Frequently, clinical researchers do not control for the severity of the disorder. Groups of "demented" subjects therefore may include patients with early as well as more advanced stages of the disease. In addition, authors seldom specify adequately the severity of the disorder in their "demented" group, thus leaving unclear, what stage of the disease is associated with particular symptoms.

5. Commonly, clinical studies on the behavioral manifestations of dementia do not distinguish between dementias of different etiologies. In these studies, in the absence of clinical diagnosis, presumptive dementia cases are identified on the basis of low scores on gross measures of cognitive functioning, such as mental status tests. Results of such studies tell us very little about manifestations of specific types of dementia, such as AD.

Thus, problems with diagnostic criteria, inadequate measures and methodological flaws preclude accurate identification of early AD at present, and may limit the possibility of accurate identification for the future. Clearly, patients with "preclinical" stages of AD would not currently meet either clinical or research criteria for the diagnosis. Further, their cognitive changes would not be picked up by a standard dementia battery, even though the process of pathological neuronal changes might have already started.

## PREDICTIVE AND LONGITUDINAL STUDIES

Detection of "preclinical" cognitive changes is of the utmost importance in the early identification of patients with progressive malignant cognitive loss versus benign age- and affect-related cognitive changes. At the present time, this task can best be accomplished by means of longitudinal comparison of neuropsychological performance with baseline neuropsychological data for each individual subject. This approach enables the clinician to document relatively subtle cognitive decline in still "normal" individuals.

In a research setting, this objective requires longitudinal follow-up of a large cohort of normal elderly individuals. According to statistical prediction, 15% of the elderly cohort will develop SDAT over time, and the incidence of disease will increase with advancing age (Cummings & Benson, 1983; Khachaturian, 1985). Several recent studies have employed this research paradigm to investigate "preclinical" changes by retrospective assignment of subjects into a "preclinical" group. Results of these studies are summarized below. Although not conclusive at this time, they show

promise in detecting "preclinical" cognitive changes associated with AD.

A prospective study which purported to tap memory deficit in a group of initially non-demented elderly employed the Fuld Object Memory Evaluation (OM) (Fuld, 1980; Fuld, 1981; Fuld, Masur, Blau, Crystal, & Aronson, in submission). In the abbreviated version of this test, 10 common objects hidden in a bag are to be identified by tactile perception, followed by visual presentation, if necessary. After 60 sec distraction period, the subject is to recall 10 objects recently identified, with selective reminding of omitted items. After an additional 10 min distraction period, another recall trial is administered, followed by recognition testing. Fifteen percent of the original cohort of non-demented subjects participating in this study developed dementia over several years. Their performance on the OM at the last annual testing before onset of cognitive decline, was retrospectively evaluated. Results suggested that the predictive validity of the test with a cutoff of 5 or fewer items recalled, was 59 percent, which is almost four times the base rate, with recall on trial 1 being the best predictive measure. Discriminative validity of the test in distinguishing normal from dementing individuals at the time of diagnosis was .86, and specificity was .82. Thus, the OM test was useful in early identification of dementing individuals well over a year before change in cognitive functioning was noted clinically.

In another prospective study, Masur, Fuld, Blau, Crystal, & Aronson (in submission) assessed preclinical changes in memory functioning through prospective testing of large cohort of normal elderly, of which some individuals later developed AD. Indices of primary and secondary memory were measured with the Selective Reminding Test (Buschke & Fuld, 1974; Hart, Kwentus, Hamer, & Taylor, 1987; Masur, Fuld, Blau, Levin, Thal, & Aronson, in press). In previous studies, this test proved to discriminate well between normal or depressed elderly and AD victims (Hart et al., 1987; Masur et al., in press). This test assessed recall of a word list over six trials. After each trial, the subject was reminded only of the words that were not recalled on the previous trial. The authors hypothesized that items recalled immediately after reminding are retrieved from primary memory, while those recalled on two consecutive trials without reminding, are retrieved from secondary memory. The test allows the evaluation of acquisition, retention and retrieval of verbal information. In addition, delayed recall and delayed recognition of the items were examined after a five minute period of distraction. This design allowed for an estimate of the consistency of retrieval (Fuld, 1980, 1981) and number of intrusions. Masur et al. (1989) found that among the above indices, measures of secondary memory, such as sum of recall and delayed recall, were the most sensitive to the emerging preclinical changes associated with development of AD.

In an earlier report looking at mental status change in the same cohort, Katzman, Aronson, Fuld, Kawas, Brown, Morgenstern, Frishman, Gidez, Eder, & Ooi (1989) followed, over a five year period, a cohort of 434 nondemented volunteers between 75 and 85 years of age on a number of psychiatric, neuropsychological, physical measures and laboratory tests. Fifty-six subjects developed a progressive dementia (with a high prevalence of females over the age of 80 in this group). Among these, 32 subjects met

diagnostic criteria for AD. The authors concluded that the major predictor of the development of AD was the mental status score on entry. Those subjects who made zero to two errors on a 33-item mental status test had a less than 0.6% per year chance of developing AD, whereas those with five to eight errors developed AD at a rate of over 12% per year. Results of this study suggested that subjects who made more than three errors on the Blessed Mental Status examination on entry, were in the "preclinical" stage of AD, thus constituting a group at high risk for subsequent cognitive deterioration.

Another type of "high risk" design was used by La Rue, Matsuyama, McPherson, Sherman, & Jarvik (in submission) who longitudinally followed siblings and children of patients with probable AD over retest intervals averaging four years and subsequently two years, on a number of psychiatric and neuropsychological measures. Individuals who showed greater than average decline from the first to the second testing were compared to those who maintained stable levels of cognitive competence. The former subjects were older, revealed higher performance at the initial evaluation (which in part might be associated with subsequent regression to the mean) and the average age at which their relatives had first shown signs of dementia was 10 years younger. The latter finding is consistent with recent literature (Huff, Auerbach, Chakravarti, & Boller 1988; Zubenko, Huff, Beyer, Auerbach, & Teply 1988) and supports the possible existence of a genetic mechanism in the development of AD. Although conclusive inferences regarding the discriminating power of different cognitive measures will be made after additional longitudinal assessments, this study raised number of interesting questions about prodromal signs of dementia and the role of family history variables as potential moderators of normal aging changes.

A similar type of prospective study was used by La Rue and Jarvik (1987) to examine longitudinal changes in a sample of 64 survivors from the larger cohort of aging twins tested 20 years previously. Individuals who developed dementia by the age of 85, had achieved lower scores on most tests twenty years prior to the diagnosis, and experienced greater declines in vocabulary and forward digit span over time in comparison to those surviving to a comparable age without dementia. The authors concluded that dementing illness may develop very slowly, and that the likelihood of exhibiting clinically significant dementia may vary with premorbid intellectual level.

Thus, identification of early "preclinical" changes associated with malignant cognitive loss versus benign age- and affect-related changes, as well as differentiation of AD from dementias of other etiologies can be accomplished by longitudinal follow-up of the progression in cognitive decline and physical symptoms. This approach allows assessment of rate and pattern of deterioration in comparison to the baseline obtained at an earlier time, thus, providing control for effect of pre-existing factors on the pattern of cognitive change.

Such longitudinal comparison can be efficiently accomodated in research; however, in clinical settings, the welfare of the patient frequently depends on accurate diagnosis based on the data gathered at one point in time. In this case, diagnostic decision-making can be aided by thorough assessment of the cognitive deficit pattern,

with special attention to the domains which might be particularly associated with the pathology and neurochemistry of AD.

## EXPERIMENTAL STUDIES OF MEMORY AND COGNITION

The most consistent finding in the early stages of AD is memory deficit. However, it is well known that many non-demented elderly people complain of forgetfulness, and that many other disorders, both reversible and irreversible, are associated with memory impairment.

### Benign Senescent Forgetfulness

Attempts to differentiate between normal memory changes due to advancing age and malignant memory deterioration associated with AD have focused on qualitative differences in the clinical picture of memory deficits induced by these two conditions. Kral (1962) introduced the notion of benign senescent forgetfulness (BSF), which describes memory changes associated with normal aging as an inability to recall relatively unimportant details of experience, such as name, place, or a date from the remote past, whereas the experience per se can be recalled. These details can be recalled at another time, which indicates deficient retrieval, rather than faulty encoding mechanisms. In addition, the person is aware of these difficulties and is trying to compensate. In contrast, malignant senescent forgetfulness was described as an amnestic syndrome with markedly impaired recent memory, gradually increasing loss of remote memory, confabulations and disorientation.

Later studies support this description by showing that the most remarkable differences between BSF and malignant memory loss are observed on measures of free recall and recognition. While normal elderly subjects demonstrate some difficulty with free recall, their performance improves remarkably on recognition tasks (Schonfield, & Stones, 1979; McCarthy, Ferris, Clark, & Crook, 1981). In contrast, immediate and delayed recall as well as recognition proved to be impaired in AD patients (Miller, 1977; Miller & Lewis, 1977; Branconnier, Cole, Spera, DeVitt, 1982; Masur, Fuld, Blau, Crystal, & Aronson, in submission; ). Evaluation of the predictive value of recall and recognition tasks as diagnostic screening tools has revealed predictive accuracy of 11% and 100% respectively (Branconnier et al., 1982).

### Differences Between Memory Disturbances in AD and Normal Aging

Studies concerned with other aspects of memory are consistent with the above observations, in that, even at the early stages of the disorder, patients demonstrate problems in several aspects of memory functioning, which are not characteristic of the pattern of normal aging. A memory deficit in AD can be observed in primary, secondary, and tertiary compartments; however, the extent of disruption in these memory compartments varies.

Impairment in primary or immediate memory can be demonstrated on several neuropsychological measures. A word generation task (verbal fluency), which requires a self-directed search of semantic memory for items from a category given by the experimenter, presents remarkable difficulties to early AD subjects, while performance of normal elderly people and depressed patients on this task is relatively intact (Rosen, 1981; Martin & Fedio, 1983; Storandt, Botwinick, Danziger, Berg, & Hughes, 1984; Flicker, Ferris, Crook, Bartus, & Reisberg, 1985; Eslinger, Damasio, Benton, & Van Allen, 1985; Morris & Baddeley, 1988). Miller (1984)

reported deficient performance of AD patients on a task requiring generation of words starting with given letter. Similarly, AD patients examined by Weingartner and coworkers performed much more poorly in comparison to normal controls on the test assessing ability to generate words that start with a given letter or that belong to a given category (Weingartner, Kaye, Smallberg, Ebert, Gillin, & Sitaram, 1981). The authors concluded that in spite of a relative preservation of many intellectual functions in the early stages of dementia, the patients are unable to access structures in semantic memory and therefore fail to effectively encode episodic events.

A similar encoding deficit is observed on other primary memory tasks. Findings by Morris and Baddeley (1988) agree with other studies in that AD subjects demonstrate a slight reduction in recall of recent items from a freely recalled list (Miller, 1971; Wilson, et al., 1983; Martin, Brouwers, Cox & Fedio, 1985; Spinnler, Della Sala, Bandera, & Baddeley, in press). AD subjects also demonstrated a mild to moderate decrease in immediate memory span for digits, letters, words and Corsi Blocks (which is a visual analogue of the memory span test) (Miller, 1977; Kaszniak, Garron, & Fox, 1979; Corkin, 1982; Morris, 1984; Flicker, Ferris, Crook, Bartus, & Reisberg 1985; Kopelman, 1985). The most pronounced difficulties in Morris and Baddeley's study (1988), however, were demonstrated by AD patients on a divided attention task (Peterson & Peterson, 1959), which requires retrieval of three verbal items after short periods of distraction with backward counting by 2s or 3s starting from a three-digit number. Whereas degree of impairment was positively related to the severity of AD (Morris & Baddeley, 1988), even very mildly demented patients showed a moderate to severe deficit on this task (Corkin, 1982). Interestingly, early-onset patients revealed greater impairment on this task, as well as on immediate recall of the sentences explored in another study (Kopelman, 1985; Kopelman, 1986b).

The above findings suggest that impairment in performance on divided attention tasks (even if each task is well within the scope of the patient's competency and addresses a modality which is not employed in the other task), is highly indicative of early changes in primary memory due to AD. Unlike digit span and free recall, this task requires simultaneous processing of competing information, which necessitates divided attention. According to Baddeley's (1983, 1986) hypothesis, inability to perform this task is due to a deficiency in the Central Executive System (CES), which controls working memory and causes disruption in nonroutine behavior, i.e. maintenance and manipulation of information. Consistent with this hypothesis, a distractor task causes excessive load on the CES, which, in case of AD, fails to optimally coordinate resources for competing tasks and insure proper maintenance rehearsal. The extent of the deficit in recall increases as a function of demands imposed by the distractor task, however; even a relatively simple task such as tapping (Morris, 1986) might cause significant impairment in recall of verbal information. In addition to disrupted performance on divided attention tasks, which directly reflect functioning of the Central Executive System, a deficient CES mechanism causes impairment in maintaining information in primary memory, as well as in retrieval of information from semantic memory (Becker, 1987; Becker, 1988; Morris & Baddeley, 1988). Thus, deficient performance on digit span and verbal fluency tasks reflects the effect of CES

dysfunction on cognition (Becker, 1988). Morris & Baddeley (1988) stated that the CES deficiency hypothesis "would incorporate an encoding deficit as one specific example of a more general impairment" (p. 292).

The relationship between primary and secondary memory deficits can be explored by comparing performance on different parts of the free recall task. Whereas only slight attenuation of the recency effect is evident in AD patients, the primacy effect is negligible or totally absent (Miller, 1971, 1977; Wilson, Bacon, Fox, & Kazniak, 1983). Miller (1971) hypothesized that this is the case because items at the end of the list are stored in primary memory, while items from the beginning of the list are transferred into secondary or recent memory.

Wilson, Bacon, Fox & Kaszniak, (1983), separately investigated primary and secondary memory components of free recall in normal and demented subjects with a technique proposed by Tulving & Colotla (1970), on which the Buschke (1973) selective reminding paradigm also depends. Free recall of four 12-word lists with a 30-sec interlist interval was tested. If the number of items intervening between presentation and recall was equal to or less than six, the item was considered to be recalled from primary memory (PM). If the number of intervening items was greater than six, retrieval from secondary memory (SM) was considered. Although some problems with SM have been documented in normal elderly (Craik, 1977; Fozard, 1980; La Rue, 1982), these problems are independent of PM functioning, as evidenced by the statistical independence of PM and SM measures in Wilson et al.'s (1983) study. In contrast, these two memory components were correlated in the group of demented subjects, which suggests that SM deficits in AD may, at least in part, be related to PM dysfunction.

Another finding consistent with the above hypothesis is the lack of proactive interference in Wilson et al.'s (1983) AD group. Whereas intrusion errors demonstrated by the normal controls were primarily items from previously presented lists, intrusions produced by AD patients were predominantly extralist items. The authors attributed this lack of proactive interference in the AD subjects to an initial processing failure, i.e. deficit in PM.

A different research paradigm in the study of proactive interference was described by Cushman, Como, Booth, & Caine (1988). In their experiment, items presented in consecutive lists, with the exception of the last list, were drawn from the same semantic category as items included in the first, free-recalled list. Sensitivity to the semantic aspect of the information is evidenced in this research paradigm by enhanced recall of the items from the last list, due to the change in semantic category, i.e. release from proactive interference (Craik & Birtwistle, 1971). The AD patients in this study failed to develop proactive interference and, consequently, failed to demonstrate release from proactive interference. The authors concluded that this failure is due to impaired ability to spontaneously utilize semantic information in encoding.

The design utilized in the above study overcomes some methodological limitations associated with free-recall tasks. A number of researchers have questioned the interpretation of deficient performance on semantic memory tasks as manifestation of

disruption in semantic memory itself (Neely, 1977; Nebes, Martin, Horn, 1984; Jorm, 1986; Nebes, Brady, & Huff, 1989). They argued that task demands might exceed attentional capacity of the patients, which presumably leads to a poor performance. To explore this possibility, it was proposed that efficient retrieval from lexical/semantic memory depends on intact functioning of two mechanisms: automatic vs. attention-dependent or effortful (Goodglass, 1980). According to Nebes et al. (1989), the former mechanism is responsible for automatic spread of activation from the node in semantic memory which represents a certain concept, to other associated concept nodes in the semantic network. This mechanism presumably does not make demands on attentional processing. In contrast, the latter mechanism requires conscious search of a location in semantic memory corresponding to a semantic context created by a task. Thus, the proactive interference design utilized by Cushman et al. (1988) minimizes loading on attention-dependent component and attempts, thereby, to accurately assess semantic memory capacity.

Another technique which reduces the amount of conscious effort and, yet, provides an accurate assessment of memory storage and efficiency of encoding is cued recall (Tulving & Pearlstone, 1966; Cushman et al., 1988). In a study by Buschke (1984), initial encoding of visual information was combined with provision of semantic categories as prompts. Following a distraction task, performance of normal and early AD subjects was examined on both free and cued recall. Performance of AD subjects on free recall was much lower than that of normal controls. However, with assistance of semantic prompts, both groups were able to retrieve virtually all of the originally presented items.

Similar results were obtained by Tuokko & Crockett (1989), who used Buschke's procedure to study effect of cues on recall in groups of patients with suspected malignant memory disorders and normal controls. In normal and in the least impaired group of demented subjects, the use of semantic cues remarkably improved recall; however, more impaired patients did not benefit from provision of cues.

These findings are consistent with the results of other studies which report substantial improvement in performance of AD subjects on cued-recall tasks in comparison to free recall or recognition tasks (Miller, 1975; Morris, Wheatley, & Britton, 1983). This enhancement in recall aided by cues is interpreted by the authors as a rate of acquisition much higher than the amount of information accessible for retrieval.

The quality of encoding at the acquisition stage can be evaluated only by comparing the rate of encoding within different semantic contexts. In the studies based on Buschke's cuing paradigm, subjects were provided with corresponding semantic categories at the time of encoding which helped them to structure information at the acquisition stage. However, studies on the effect of cued recall, which did not provide a structural context at the acquisition stage, reported different results. Davis & Mumford (1984) used the initial letter of the word and its semantic category as prompts at the time of recall of word lists. No aid in semantic organization at the time of encoding was provided. The authors found that normal controls benefited from both letter and category cues, whereas demented patients were aided only by letter

cues. Improvement in recall with letter cues in AD subjects was also reported by Miller (1975) and Morris, Wheatley, & Britton (1983). Thus, provision of semantic cues at the retrieval stage in Davis & Mumford's study did not facilitate recall in AD group. This finding is consistent with results reported by Cushman et al., (1988), who also did not observe any facilitating effect of semantic cues at the retrieval stage on recall of word lists in the group of AD subjects. The authors infer that AD patients' deficits in episodic memory are related to the impairment in utilizing semantic information at the encoding as well as the retrieval stage. Cushman et al., (1988) stated:

> It appears that impaired performance on episodic memory tasks in AD (e.g., free and cued recall) may reflect co-existing deficits in semantic memory, as well as a diminution of the ability to incorporate semantic information in organizing newly learned information. Indeed, deficits in semantic processing may well underlie or precede failures in episodic memory in AD.
>
> (p. 691)

A number of other research strategies support this assumption. Wilson, Kramer, Fox, & Kaszniak (1983) compared word frequency effect on recall and recognition in normal elderly and AD subjects. AD victims showed a normal tendency to "false alarm" to common words, but they failed to show a rare word advantage. Normal controls revealed remarkably better recognition memory for rare than for common words. The authors concluded that AD patients fail to encode the featural and intrastructural elements of verbal information, which might contribute to impairment in their recognition memory.

Similarly, Miller (1981) in his review of studies on memory and cognitive functioning in senile dementia pointed out that paired associate learning in demented subjects was slow and was equally impaired on recall and recognition conditions, implicating a problem in encoding rather than retention.

A semantic memory deficit is also responsible for the failure of AD patients to demonstrate a generation effect (Mitchell, Hunt, & Schmitt, 1986). Generation effect can be demonstrated in better retention of words that have been previously generated by a subject, than of words which have simply been read by a subject. This effect occurs because, in comparison to reading, generating an item produces greater activation of its representation in lexical memory (Slamecka & Graf, 1978; McElroy & Slamecka, 1982). Consequently, inadequate representation in lexical or semantic memory in AD patients may account for the absence of generation effect.

The hypothesis of a deficit in semantic encoding underlying the disturbance in secondary memory is also supported by the inability of AD subjects to benefit from organization of presented material in a categorized list (McCarthy, Ferris, Clark, & Crook, 1981; Weingartner et al., 1981; Weingartner, Grafman, Boutelle, Kaye, & Martin, 1983; Diesfeldt, 1984), and by inefficient use of mnemonics (Butters, 1984). In a study on the effect of different encoding and retrieval conditions on rate of recall in AD patients, Diesfeldt (1984) found increase in recall when explanation of the category structure during encoding was combined with category cueing at retrieval. However, retrieval cueing with category names

without provision of the category structure did not facilitate recall. These results suggest that patients were unable to self-generate an encoding and retrieval strategy, however, they could benefit from explanations of semantic structure, provided by the experimenter.

Another research paradigm purport to explore the role of semantic context utilizes semantic priming. Studies exploring the effect of semantic priming on efficiency of information processing suggested that priming with a semantically related context facilitates processing of the stimulus. Nebes and coworkers (Nebes, Martin, & Horn, 1984; Nebes, Brady, & Huff, 1989) used word-naming and lexical-decision tasks to examine effect of semantically-related context on the identification of the stimulus in normal elderly and AD subjects. The word-naming task requires the subject to name visually presented stimulus word, while a lexical-decision task requires the subject to determine whether a given string of letters constitutes an English word (Nebes, Brady & Huff, 1989). As was shown by Seidenberg, Waters, Sanders, & Langer (1984), priming effects in a word-naming task are due to the automatic spread of activation, while those in a lexical-decision task reflect attention-dependent processes, as well. Nebes and co-workers showed that in both studies (1984, 1989) AD patients revealed an equal or substantially larger priming effect (i.e. the difference in response time to a word precipitated by an unrelated vs. a semantically related word) on both tasks, in comparison to the normal controls. However, lexical access time was slower for the group of demented patients. The authors concluded that AD patients benefit from semantic context whether it is mediated by automatic or attention-dependent processes.

Following Jacoby's (1984) model, Nebes et al. (1989) hypothesized that the principal distinction in memory functioning between normal and AD subjects stems not from differences in an attention-dependent mechanism, but from the efficiency of intentional vs. incidental retrieval. With incidental retrieval, cues are provided by the stimulus material and task situation themselves, while with intentional retrieval, cues are self-generated by the subject. Based on this framework, Nebes et al. (1989) made the following assumption:

> demented patients should be most successful on tasks in which their access [to] and use of semantic information is heavily constrained and guided by the task and stimuli (e.g., by the presence of pre-existing semantic association). In contrast, they should have a great deal of difficulty on tasks that require a self-directed search of semantic memory. (p. 229)

Thus, the above studies which utilized diverse techniques and addressed different aspects of secondary memory, consistently indicate that the deficit in secondary memory stems from an inability to self-generate an encoding strategy and, therefore, to successfully access semantic memory.

Although secondary memory appears to be the most vulnerable to the dementing process, some AD-related changes can be observed in tertiary or remote memory. These deficits also result from impaired access to lexical-semantic knowledge (Becker, 1988), as revealed by difficulty with visual confrontation naming (Bayles, 1982; Bayles & Tomoeda, 1983; Kirshner, Webb, & Kelly, 1984; Huff, Corkin, & Growdon, 1986; Martin, Brouwers, Lalonde, Cox, Teleska,

Fedio, Foster, & Chase, 1986) and by altered memory for remote public events (Wilson, Kaszniak, & Fox, 1981).

Huff et al. (1986) showed that naming difficulty in subjects with mild to moderate AD is related to semantic impairment causing difficulty in distinguishing among objects that are members of the same semantic category. This difficulty may also partly underlie the presence of intrusions in the data reported by Fuld et al. (1982), and also of clinical obsevations of "poor judgment" in AD patients. It was also argued that a semantic deficit occurs independently of visuospatial dysfunction. Huff et al. (1986) found that the frequency of errors made by AD patients in a confrontation naming task was similar to that of a name recognition task (which requires a response on whether the name presented matched a projected picture of the object). This finding led the authors to the conclusion that specific semantic information is unavailable to both automatic and deliberate (attention-dependent) semantic processes. On the other hand, Flicker et al. (1985) showed that the performance of subjects with mild to moderate AD on the task which requires pointing out named objects, is not significantly different from that of normal elderly. The relationship of task difficulty to stage of dementia may be crucial in reconciling discrepant findings such as these. Nevertheless, deficits in tertiary memory appear to point to a common mechanism underlying disruption in other memory compartments. Whereas difficulties revealed on confrontation naming tasks did not indicate general disruption of semantic processing, they pointed to specific deficits in access to lexical/semantic knowledge.

Above findings support a two-component model of memory deficit in AD, proposed by Becker (1988). According to this model, an encoding deficit associated with dysfunctional CES mechanism of primary memory results in an inability of AD patients to self-generate an encoding strategy which would facilitate access to information stored in semantic memory. This primary memory disfunction underlies deficits in all memory compartments. Another component of memory loss is deficit in secondary memory, manifestations of which are similar to an amnestic syndrome and can be related to impairment in episodic memory (Moscovitch, 1982; Cermak, 1984; Morris & Kopelman, 1986; Becker, 1988). It is marked by an inability to benefit from encoding or 'cognitive mediation' during learning (Warrington & Weiskrantz, 1982; Cermak, 1986). In view of this hypothesis, the deficit in retention is due to an inability to remember the encoding process itself, rather than to a deficiency in stimulus encoding (Becker, 1988).

## RELATING EXPERIMENTAL FINDINGS TO PATHOLOGICAL AND NEUROCHEMICAL CHANGES

It is possible to relate the experimental findings described above to findings from neuropathological studies. According to Perry and Perry (1982), areas primarily affected by disease process are the hippocampi, the temporal and the frontal lobes. The secondary memory deficit can be related to the pathology in temporal areas and in the hippocampi which disrupt hippocampal-cortical connections (Hyman, Van Hoesen, Damasio, & Barnes, 1984; Hyman, Van Hoesen, Kromer, & Damasio, 1986). In contrast, the primary memory, and encoding strategy impairments can be associated with the frontal pathology (Shallice, 1982; Baddeley & Wilson, 1988). Based on neuropathological studies, several subcortical structures are also affected by the disease process, among which

nucleus basalis of Meynert has especial implications (Candy, Perry, Perry, Biggins & Thomson, 1983; Saper, 1986). This structure has numerous projections into frontal cortex. Loss of its neurons could result in deficient control processes and might account for "frontal lobe" features such as poor judgment and erosion of semantic discriminative ability demonstrated by AD patients (Becker, 1988).

One of the manifestations of deficient control exerted by the frontal lobes is the phenomenon of disinhibition (Warrington & Weiskrantz, 1970), which results in inability to suppress irrelevant information in the search for correct response. In light of this hypothesis Miller (1981) argued that provision of partial information (such as cued recall, etc.) enhanced recall by giving cues for the correct response and limiting the alternatives to be recalled.

The disinhibition hypothesis is also consistent with intrusions of irrelevant material observed in AD patients (Fuld, 1980). In fact, a number of studies have suggested that the frequency of intrusions can be viewed as a marker of cholinergic deficiency associated with AD (Fuld, Katzman, Davies, & Terry, 1982; Brinkman & Gershon, 1983; Brinkman, Largen, Cushman, Braun, & Block, 1985; Fuld, 1985). According to Fuld et al. (1982) an increase in the frequency of intrusion errors is negatively correlated with levels of cholinergic enzyme choline acetyltransferase in the brain, and is positively correlated with the number of neuritic plaques in the cerebral cortex on the autopsy. The hypothesized relationship between frequency of intrusion errors and central cholinergic deficiency is supported by pharmacological (cholinergic) models, which reveal AD-like changes in cognitive functioning in non-demented people in response to scopolamine treatment, which blocks cholinergic neurotransmission (Drachman & Leavitt, 1974; Brinkman et al., 1985; Fuld, 1985; Beatty, Butters, & Janowsky, 1986). In addition, in a study on manipulation of an anticholinergic drug with concurrent evaluation of memory functioning by the Buschke-Fuld Selective Reminding Test, the number of intrusion errors was related to drug level (Thal, Fuld, Masur, Sharpless, & Davies, 1983).

The type of intrusion errors described in these studies was somewhat different from the extralist intrusions described by Wilson et al. (1983), (see above). Fuld et al. (1982) defined intrusion errors which were observed on the Fuld Object-Memory Evaluation (Fuld, 1981), the Blessed Mental Status Test (Fuld, 1983) and WAIS-R subtests, as perseverations, i.e. "the inappropriate recurrence of a response (or type of response) from a preceding test item, test, or procedure" (Fuld, 1985, p. 304). Although this is a commonly recognized characteristic sign of frontal lobe pathology (Luria, 1966), the phenomenon also involves poor judgment and poor discrimination. Similarly, the perseverative nature of such responses early in the course of AD is evident on a continuous-recognition task, in which the subject is to respond whether or not he has previously seen the stimulus (Albert & Moss, 1984). This tendency for intrusions and perseverations is much greater in AD patients, in comparison to normal aging or dementias of other etiology. For example, Brinkman et al. (1985) reported a higher frequency of intrusion errors in AD patients than in MID patients. Thus, the tendency to produce

intrusions and perseverations lends further credence to the hypothesis of frontal cortex involvement in AD.

The above findings support a two-component model of memory loss in AD and provide better insight into mechanisms underlying the subtle memory deficits associated with early AD.

An impressive body of knowledge is accumulated on non-mnemonic cognitive impairments which are characteristic of Alzheimer's disease. Although these deficits are thought generally to develop later in the course of the disease, there may be some exceptions.

In addition to the most common group with early memory impairment, clinicians encounter at least two groups of relatively atypical patients. One type demonstrates progressively increasing language impairment, similar to an aphasic disorder and showing less dramatic memory changes. Several studies suggest that this type predominates in presenile dementia with onset of symptoms before age 65 (Seltzer & Sherwin, 1983; Filley, Kelly, & Heaton, 1986). The other type presents with remarkable visuo-spatial deficits as the first symptoms of AD with secondary impairment in other cognitive domains (Albert & Moss, 1984). According to Albert & Moss (1984), this heterogeneity in presenting symptoms might be due to different combinations of neuropathological or neurochemical loss. The authors speculate that at least one of the varieties in the presenting symptoms may be due to the presence of both cholinergic and catecholaminergic deficits. Further studies are needed to explore this possibility.

A marker which may be sensitive to early impairment is that of Fuld (1984b), who suggested that a certain relationship between WAIS or WAIS-R subtests is characteristic of AD, in contrast to other dementias. She developed a profile for differentiation of AD cases from other dementias based on age-corrected scores on 7 WAIS subtests:

$$A>B>C\leq D,\ A>D,$$

where A is the mean of the Information and Vocabulary subtest scores, B of the Similarities and Digit Span, C of the Digit Symbol and Block design, and D is the Object Assembly score. Application of this profile to different study samples revealed high discriminative validity. Similar results were obtained by Brinkman and Braun (1984), who showed that the Fuld WAIS-R profile observed in 56% of AD subjects, was evident in only 5% of the MID subjects. Tuokko and Crockett (1987) investigated the frequency of this WAIS-R profile in a sample of 74 healthy elderly subjects. This frequency proved to be very low (only 1%). A similar design was used by Satz, Van Gorp, Soper & Mitrushina (1987) on a larger sample of 149 healthy elderly volunteers between ages 60 and 94, of which 13% manifested WAIS-R profile. To explore the frequency of the profile across different ages, Satz and coworkers examined the incidence of this AD marker in the national standardization sample for the WAIS-R battery (Satz, Hynd, D'Elia, Daniel, Van Gorp, & Connor, in submission). The results revealed a steady increase in frequency of the marker over age, from .5% in the 16-17 age group to 36.25 in the 70-74 age group, based on reference-group scaled scores. The authors concluded that the increase in incidence of the WAIS-R profile demonstrates an effect of aging as observed in the WAIS-R standardization sample, and may reflect the presence of an increasing proportion of people showing signs of AD (Satz et al., in submission). It may also reflect the presence of frontal

lobe dysfunction from other causes. Therefore, at this time, causes of a linear progression in frequency of the WAIS profile in the WAIS-R standardization sample can not be defined as 'normal' or 'pathological'. In addition, the results revealed significant sex and age effects on frequency of the profile. This might account for controversial differences in base rates between different studies. The above findings support the possible potential of the WAIS-R profile in early identification and differential diagnosis of AD. However, further evaluation of its clinical utility is essential because inferring AD from the presence of a test profile in a community sample carries with it a high risk of false positives.

The fact that a number of other symptoms, such as deterioration of visuo-spatial ability, language, sensory and motor systems usually occur later in course of the disease would seem to raise a question about the likelihood that the Fuld profile would occur in early cases, despite these findings. It may be that more study of the probability of false positives, or positives from causes other than AD, will clarify this. A prospective study would be most revealing.

## NON-COGNITIVE CHANGES

In search of an early marker of AD, we should not overlook the non-cognitive element which affects overall performance on the task. It is well known that most measures of memory depend on both the ability and the willingness of the subject to respond. The latter includes the subject's affect and level of motivation. There is still little research done on distinguishing cognitive and non-cognitive components of the memory deficit in AD, primarily due to a lack of a reliable instrument which would measure non-cognitive changes associated with AD. Two approaches in assessment of affective status are usually used in clinic. According to one approach, the patient rates his affect on a series of items (e.g. Zung Self-Rating Scale for Depression). The second strategy involves rating of patient's affect by physician (e.g. Beck Depression Inventory). Both of these approaches are prone to errors. Cognitive changes in dementing patients jeopardize the reliability of their self-rating. Furthermore, physician's ratings are in large part based on the patient's self-perception and reporting, which might also be inaccurate.

Clinical observations suggest that the best informants regarding subtle changes in personality and affect of AD victims are their significant others. The first attempt to develop an instrument which taps personality and affective changes as they are perceived by patients' relatives was undertaken by Satz and coworkers. The Neuropsychology Behavior and Affect Profile (NBAP), which consists of 5 clinical scales, is undergoing validation (Nelson, Satz, Mitrushina, Van Gorp, Cicchetti, Lewis, & Van Lancker, in print). Preliminary results suggest that the NBAP may have potential for discriminating between non-cognitive changes associated with AD and affective fluctuations expected in normal elderly. How early such change become detectable is yet to be determined.

## RECOMMENDATIONS FOR FUTURE RESEARCH

In this chapter we have explored the research which lays the groundwork for the possibility of early, or pre-clinical diagnosis of typical cases of AD. Because, in most cases, memory impairment

is the earliest and most consistent finding in AD, we have limited our review primarily to studies of memory-related functions. Despite the general wisdom that non-mnemonic function may become impaired later in the course, the work of Fuld (1984b) and of Satz and his colleagues (Satz et al., in submission; Nelson et al., in print) both suggest that other domains of tests would seem to warrant further study.

The numerous experimental studies which show differences in specific cognitive or mnemonic functions between subjects with AD and normal or depressed elderly only provide the first step in developing the clinical instruments which are needed for pre-clinical detection of dementia. Additional prospective studies employing promising tasks must document the predictive validity of each new measure or combinations of measures or profiles, and studies must be done to clarify the frequency and consequences of overdiagnosis (false positives) at the preclinical stage. If this work is neglected, we may see an era of "iatrogenic pseudodementia" or premature exacerbation of morbidity in early actual cases.

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# Neuropsychology:
## Normal Aging versus Early AD

Ronald F. Zec
*Department of Psyciatry and Regional Alzheimer Disease Center, School of Medicine, Southern Illinois University, Springfield, IL*

**INTRODUCTION**

NINCDS-ADRDA criteria for the clinical diagnosis of PROBABLE Alzheimer's disease (AD) requires that the dementia be established by clinical examination, be documented by the Mini-Mental Test or some similar examination, and be confirmed by neuropsychological tests (McKhann et. al., 1984). There must be deficits in two or more areas of cognition, progressive worsening of memory and other cognitive functions, e.g., language, praxis, attention, problem-solving, visual perception. The main question to be addressed in this paper is whether a neuropsychological evaluation can reliably distinguish normal aging from early dementia of the Alzheimer type.

The differential diagnosis of early dementia versus aging is a challenge since normal aging can be accompanied by declines in memory and other cognitive functions (Parsons and Prigatano, 1978) and dementia of the Alzheimer type begins insidiously. Furthermore, the detection of early Alzheimer dementia has been made more difficult due to the lack of elderly normative data (Heaton, et. al., 1986; McKhann et. al., 1984). Normative data are virtually non-existent for the so-called "old-old" groups (age 75 and above) who make up the majority of the referrals to our Regional Alzheimer Disease Center. The effects of aging versus early dementia of the Alzheimer type on cognition and neuropsychological test performance will be discussed and recommendations will be made for how the early detection of Alzheimer dementia can be improved.

**EFFECT OF AGING ON COGNITIVE ABILITIES AND NEUROPSYCHOLOGICAL TEST PERFORMANCE**

<u>The Effects of Normal Aging on Cognitive Abilities</u>

A diagnosis of dementia must be made in the context of normal aging since dementia typically occurs in the elderly.

Thus, an understanding of the cognitive and memory changes which are associated with normal aging are a prerequisite to both the understanding and differential diagnosis of dementia.

Different findings with regard to the effects of aging are obtained depending on whether a cross-sectional or a longitudinal research strategy is used. The cross-sectional method compares different age-groups at one particular point in time. The longitudinal approach does repeated evaluations of a sample of subjects over time. The cross-sectional approach reveals an accelerating decline in mental abilities, e.g., intelligence, with increasing age (Wechsler, 1981; Miller, 1977).

Longitudinal studies, on the other hand, have suggested cross-sectional studies overestimate the rate of decline with age (Miller, 1977). One study using the Army Alpha test reported a slight non-significant trend for subjects tested at age 50 and again 11 years later (Owens, 1966). Schaie and Strother (1968a; 1968b) combined both methods by administering the primary Mental Abilities test to cross-sectional samples of subjects at 5-year age intervals from 20 to 70 years and then retesting each subject 7 years later. The results of this study indicated that cross-sectional data overestimated age declines except where speed was a factor.

Both education and nutrition have been reported to affect performance on intelligence tests (e.g., Husen, 1951). Differences in quality of education, nutrition etc. are confounding factors which may have more to do with the cohort differences found in cross-sectional studies than age per se. Thus, although intellectual performance declines with age especially at older age levels, this decline is not as large as once was thought (Miller, 1977).

The causes of age-related changes are no doubt mulifactorial. Sensory deficits, general health, motivation, attitudes are among the many factors which may affect cognitive test performance other than aging per se. For example, hypertension is associated with lower scores on intelligence testing (Wilkie and Eisdorfer, 1971).

Different cognitive abilities decline at different rates with advancing age, e.g., verbal skills and stored information tend to remain relatively intact while perceptual-integrative and psychomotor skills deteriorate the most (Botwinick, 1967). The normative data on the WAIS shows that some subtests are more susceptible to decline with age (i.e, "don't hold" subtests: Digit Symbol, Block Design, Digit Span, and Similarities) than others (i.e., "hold" subtests: Vocabulary, Information, Object Assembly, and Picture Completion) (Matarazzo, 1972). Subtests sensitive to aging place particular emphasis on memory or novel problem-solving. The subtests that are less affected by aging emphasize acquired knowledge.

Schaie and Strother (1968b) found that Verbal Meaning, Number, Space and Reasoning started to show appreciable decline in sixth decade, while word fluency (a timed test) started to decline much earlier. In an experiment in which subjects were required to sort cards according to whether a card contained an

"A" or "B," the addition of irrelevant letters had a greater negative effect on the speed of older subjects (Rabitt, 1965). Schaie and Gribbin (1975) argue that the age at which decline starts to be evident is getting later with succeeding generations.

A widely used conceptual framework for understanding age declines in ability is the "fluid" versus "crystallized" intelligence distinction (Cattell, 1943). "Fluid" intelligence refers to the ability to acquire new concepts and to adapt to new situations. "Crystallized" intelligence refers to learned intellectual skills. Evidence suggest that fluid intelligence is more affected by aging than crystallized abilities (Savage, 1973). The finding that the correlation between performance on Raven's Progressive Matrices (fluid ability) and the WAIS Vocabulary subtest (crystallized ability) was higher in young adults than in elderly subjects (Cunningham et. al., 1975) is consistent with this notion.

Most studies of the effects of aging on memory have been guided by the linear information-processing model (Kaszniak, et. al., 1986). According to this model, information flows through a series of stages (sensory, primary, secondary and tertiary memory) and difficulty in any stage will result in a "bottleneck" and impair memory (Atkinson & Shiffrin, 1968). Sensory memory is a preattentive and very unstable, e.g., iconic memory measured by the backward masking paradigm. Primary (short-term) memory is a temporary, limited-capacity store and can be measured using a foward digit span test. Secondary (long-term) memory refers to a more permanent store for newly acquired information. Tertiary (remote) memory refers to the store of well-learned information. Studies have shown that speed of retrieval from each of these theoretical memory stores declines with age (Fozard, 1980; Poon, 1985; Kaszniak et. al., 1986). Studies also demonstrate that normal aging mainly effects the acquisition and retrieval of new information from secondary memory, while the capacities of sensory, primary and tertiary memory are unaffected (Fozard, 1980; Kaszniak et. al., 1986).

Declines in secondary memory with aging could be due to a failure of acquisition, storage and/or retrieval. Evidence indicates that the impairments in secondary memory as a function of aging are predominately due to problems in encoding and retrieval rather than storage (Kaszniak, et. al., 1986). Rates of forgetting (storage efficiency) for a variety of different memory paradigms have not been found to be different in young and old adults. Nor has proactive and retroactive inhibition been shown to differ.

Encoding and retrieval are difficult to evaluate independently (Kaszniak et. al., 1986). Encoding deficits have been inferred from studies which have demonstrated a significant age-related deficit in learning and later recall when the material to be learned requires verbal elaboration, visual elaboration or organization (Smith, 1980). Some of these secondary memory deficits in the elderly can be greatly reduced by teaching elderly individual memory strategies (Poon, Walsh-Sweeney, & Fozard, 1980; Treat, Poon, & Fozard, 1981).

Retrieval as opposed to storage deficits have been inferred from the well-established finding that aging has small effects on recognition memory but large effects on recall, which requires active memory search and retrieval (Schonfield and Robertson, 1966). The normalization of performance in the elderly with cueing also points to a retrieval deficit rather than a storage deficit. In one study younger and older subjects were asked to try to remember a list of 36 words which fell into one of six categories (Laurence, 1967). Being given these category cues during the learning trial had no effect on the recall of either group. When these cues were given at the time of recall, performance of the older subjects improved to the level of the younger subjects. This finding suggests that the older subjects have particular difficulty with retrieval.

Reaction time decreases throughout adult aging (Botwinick, et. al., 1957) and it appears to be due to central rather than peripheral changes. Varying the intensity of the stimulus or the size of the motor pathway involved in the response have little effect on response speed in elderly subjects (Botwinick, 1971; Birren and Botwinick, 1955). On the other hand, elderly subjects have difficulty with long and short preparatory intervals between the warning and reaction-time stimuli but not with intermediate intervals (Botwinick and Thompson, 1966). The elderly appear to have difficulty maintaining expectancy or set for long periods and learning how to quickly prepare to respond (Botwinick, 1973).

It is important to keep in mind that there are many possible contributing factors to the age-related changes in memory and cognition. Sensory deficits, motivation and general health are among the many factors which may affect cognitive test performance other than aging per se. For example, hypertension is associated with lower scores on intelligence testing (Wilkie and Eisdorfer, 1971).

## The Lack of Adequate of Age Norms for Neuropsychological Tests

The report of the NINCDS-ADRDA Work Group states that a diagnosis of PROBABLE Alzheimer's disease must be confirmed by neuropsychological tests (McKhann et. al., 1984). The comprehensive neuropsychological screening evaluation for dementia which we use at the Southern Illinois University School of Medicine assesses a broad range of cognitive functions including general intellectual functioning, verbal abstraction, judgment, attention, memory functions, visuo-spatial and constructional abilities, verbal fluency, and confrontational naming ability. The neuropsychological tests in our battery are tests that have been recommended for use in the assessment of patients with suspected Alzheimer's disease by the NINCDS-ADRDA Work Group , and other investigators (Rosen, 1983a, 1983b, Kaszniak, 1986). The report of the NINCDS Work Group also states that "because there are no normative population standards for many of these tests, abnormal performance can be determined only by comparison with a normal control group matched for age, sex, and local education (McKhann et. al., 1984)."

The incidence rate for new cases of AD increases with age (Schoenberg, et. al., 1987). For example, the incidence in Rochester, New York from 1960-1964 was 3 for ages 50-59, 14 for

ages 60-69, 48 for ages 70-79, and 52 for ages 80-89 (Schoenberg, et. al., 1987). Furthermore, it is also well-established that scores on neuropsychological tests can be significantly affected by the age and educational level of the subject (Parsons and Prigatano, 1978; McKhann et. al., 1984). Therefore, it is particularly important to have adequate normal control data for neuropsychological tests, especially for the upper age ranges. It is clear, however, that normative data for the neuropsychological tests in our diagnostic battery are very inadequate for the elderly age ranges.

The best normative data is available for the Wechsler Adult Intelligence Scale - Revised (Wechsler, 1981) and the Wechsler Memory Scale - Revised (Wechsler, 1987), but the oldest age group included for these tests is age 70-74 years. In a letter to our research group dated 12/17/85, a representative of The Psychological Corporation said that "norms for the WAIS-R for people over 74 years would be helpful in many applications" but "it has been the Psychological Corporation's experience over the years that elderly subjects are often difficult to locate, to approach, and to test in connection with standardization projects." In the past year 57% of our suspected AD patients were age 75 or old, i.e., in an age range for which there is no published normative data available on the WAIS-R and WMS-R.

One study reported normative data for Trails A and B only for patients in the overly broad age-category of 41-64 years of age (Reitan, 1979). Furthermore, there were only a total of 10 subjects in this group. In another study Trails A and B normative data are published for patients only in the overly broad age-groups of 40-59 and 60+ years (Birren and Morrison, 1961).

The Rey Auditory Verbal Learning Test has norms for elderly patients only in the overly broad age-range of 70 to 90 years and is based on a very small number of subjects, i.e., 15 in the elderly laborer group and 15 in the elderly professional group (Rey, 1964 in Lezak, 1983). The Benton Visual Retention Test has norms for the age ranges 40-54, 55-59, and 60-64 for different IQ classifications, but no norms for patients 65 and older (Benton, 1974).

No elderly norms are available for either the "young-old" or the "old-old" for the Rey-Osterreith Complex Figure Test (Lezak, 1983). The Hooper Visual Organization Test provides conversion scores for the half-decades from 25-69 years of age stratified according to educational level, but provides no normative values for age 70 and above (Hooper, 1958).

Age norms for the current 60-item version of the Boston Naming Test are only available for the 50-59 age group and is based on only 22 subjects (Kaplan, 1983). An older 85-item version of the test has age norms for each half-decade from 60-85 years old but is based on relatively small numbers of subjects per age range, i.e., 3, 17, 23, 10, and 5 respectively (LaBarge, 1986). These authors do report, however, that naming seems to remain relatively intact over these age ranges.

Word Fluency norms are available for the letters "P-R-N" for the following age ranges: 25-54, 55-59, and 60-64 (Benton, 1978). No norms are published from age 65 and above. In addition, there are no published norms for the aged for word fluency using category stimuli, e.g., animals, vegetables and states.

There are no published norms for the version of the Stroop Test which we employ. Age-correction scores are provided for another version of the test only for patients in the overly broad age-ranges of 45-64 years and 65-80 years (Golden, 1978). Furthermore, the authors state that the age corrections for the latter group are considered experimental.

Although several neuropsychology laboratories are in the process of collecting neuropsychological control data for the normal elderly, very little has yet been published. Furthermore, it has been pointed out that regional normative-control data are needed to compare with patients drawn from the same geographical region of the country (McKhann et. al. 1984). A three-center study on neurobehavioral outcome following minor head injury reported that the median scores of the normal control group from one region of the country approximated the median scores of the brain-damaged groups from the two other regions (Levin, 1987). This is presumably because of differences in quality of education and other cultural and socioeconomic differences. The effects of differences in quality of education and related variables is also demonstrated by the finding that cross-sectional studies as opposed to longitudinal studies tend to overestimate age effects by confounding these with educational/cultural differences among groups of subjects from different generations or "cohorts" (Botwinick, 1967). Thus, adequate normative neuropsychological data on the elderly is needed and local normative data for different socioeconomic/cultural groups and geographical areas is strongly recommended.

**EARLY DETECTION OF DEMENTIA OF THE ALZHEIMER TYPE**

Since a comprehensive review of the neuropsychological characteristics of early Alzheimer's disease appears elsewhere in this volume (see Mitrushina and Fuld, 1990), only a brief summary of selected issues dealing with early detection will appear in this section. Data from our labortory will also be presented regarding the ability of three neuropsychological tests to detect early AD.

A typical neuropsychological profile for a person with mild to moderate AD includes a reduction in overall level of intellectual functioning (including deficits in judgment and abstract reasoning), a greater Verbal IQ than Performance IQ, recent memory impairment (both verbal and nonverbal), constructional problems, reduction in verbal fluency, and variable language deficits with naming difficulties being most common (Rosen, 1983a, Rosen 1983b, Kaszniak, 1986). While impaired intellectual functioning and the symptom triad of memory loss, anomia, and impaired visuospatial skills have been suggested as the hallmark of AD, patients may exhibit only one or two of these symptoms in the early stages of the disorder (Rosen, 1983a). This variability in dysfunction in the early stages of AD may lead to misdiagnosis. This underscores the necessity for a thorough assessment, for caution in diagnosis, and for

retesting twelve months after the initial evaluation to document significant changes.

A two-hour neuropsychological test battery was administered three times over 2.5 years to patients with mild Alzheimer's dementia and normal control subjects (Storandt, Botwinick, and Danzinger, 1986). The test battery included parts of the Wechsler Memory Scale (Mental Control, Logical Memory, Digit Span, Associate Learning), four WAIS subtests (Information, Comprehension, Block Design, Digit Symbol), the Benton Visual Retention Test (memory and copy), Boston Naming Test, Word Fluency, Trail Making Test Part A, Bender Gestalt and Crossing Off Test. Patients and control subjects differed significantly on all tests except Digit Span forward during the initial baseline assessment and while no significant decline was found in the control subjects over time, the patient group declined on all tests. Thus, these standardized tests differentiated patients with mild SDAT and normal age-matched control subjects. The authors conclude that secondary memory and psychomotor speed are especially sensitive to early Alzheimer dementia but that even early patients perform poorly on most psychological tests.

Memory deficits are typically the first cognitive deficits to appear in DAT, followed by problems with abstract reasoning and complex attention, and then by impairments in language and visuospatial abilities (Grady et. al., 1988). In particular, memory for events (episodic memory) is usually impaired early in the course of DAT, and this impairment becomes increasingly severe with dementia progression (Butters, 1984; Kaszniak, 1986; Grady et. al., 1988). Also, unlike normally aging individuals, DAT patients show rapid forgetting of events (Moss et. al., 1986). There have been reports of patients with Alzheimer disease, confirmed by biopsy or autopsy, presenting initially with a progressive right parietal lobe syndrome (Crystal et. al., 1982) or a progressive aphasia (Kirshner et. al., 1984). However, the overwhelming evidence to data indicates that course of DAT usually begins with memory impairment and progresses to other cognitive problems. Thus, tests of episodic memory including measures of both immediate and delayed recall should be included within any dementia assessment battery. Memory measures are among the most useful early detectors of dementia of the Alzheimer type.

Unlike the normal elderly who only show declines in secondary memory, Alzheimer dementia is associated with impairment in all stages of memory: sensory, primary, secondary, and tertiary (Kaszniak, et. al., 1986). The impairment of primary memory tends to be mild in early dementia of the Alzheimer type but progressively worsens over time. Some experiments indicate that the deficit in secondary memory is due to failure to encode essential aspects of to-be-learned information. Primary memory impairment may also limit the amount of information which is tranferred into secondary memory.

The sensitivity of a given neuropsychological test to the progressive deterioration in Alzheimer patients is a function of the patient's initial level of performance on that test (Mohs, et. al., 1986). Tests on which the patient exhibits an intermediate degree of impairment are most likely to be sensitive

to the progressive worsening in the coming year. Tests that are too difficult or too easy will not be sensitive to immediately subsequent cognitive declines. For example, although recall was more impaired on the initial evaluation, recognition memory better documented progressive worsening over 12 months (Mohs, et. al., 1986). Learning low-frequency paired associates, a difficult task, and naming common objects, an easy task, were both insensitive to change, but tasks of intermediate difficulty, including sentance reading and paired-associate learning, did show decline at one year follow-up (Mohs, et. al., 1986).

Recent data from SIU School of Medicine

We recently studied the utility of three tests in the detection of mild dementia of the Alzheimer type. Patients all met NINCDS-ADRDA criteria for PROBABLE Alzheimer's disease. Severity of dementia for the data discussed below was defined on the basis of scores on the Folstein Mini-Mental State Examination: mild = 20 and above, moderate = 10-19, severe = 0-9.

The Alzheimer Disease Assessment Scale (ADAS) was administered to 61 Alzheimer patients and 52 elderly controls (Zec, et. al, 1989). The ADAS was developed to comprehensively assess the multiple areas of cognitive impairment which typically characterize dementia of the Alzheimer type (DAT) (Rosen, Mohs, and Davis, 1984). The mild DAT group (n = 23) had a mean MMSE of 22.2±3.1 with a range of 20-26. Using a cutoff score of 3 SD below the control group mean, 100 of the elderly controls, 87% of the mild DAT and 100% of the moderate and severe patients were correctly classified. Using a cutoff score of 2 SD below the control group mean, 96% of the elderly controls, 91% of the mild DAT and 100% of the moderate and severe DAT were correctly classified. Thus, the ADAS-cognitive was highly effective in discriminating individual Alzheimer patients, including mild DAT, from elderly controls.

Phonemic word fluency (PWF) and semantic word fluency (SWF) were compared in 84 Alzheimer patients and 48 elderly controls (Zec, et. al., 1990). The word fluency test consisted of six, one minute trials. During each trial the subject had to generate words beginning with a certain letter (P, R, W, i.e., PWF) or belonging to a certain category (animals, vegetables, states, i.e., SWF). SWF was considerable better in discriminating Alzheimer patients from normal aging. Using a cutoff score of 2 SD below the mean for the control group, PWF and SWF correctly classified 100% and 96% of the controls. Using the same cutoff, hit rates for AD patients were only 50% using PWF but 92% using SWF. Hit rates for mild (n = 48), moderate (n = 26) and severe (n = 10) dementia were only 35%, 65% and 80% using PWF, but 85%, 100% and 100 using SWF. Thus, semantic word fluency but not phonemic word fluency was useful in the early detection of mild DAT.

Performance on the Rey Auditory-Verbal Learning Test (RAVLT) was compared in 86 Alzheimer patients and 53 elderly controls (Zec and Vicari, 1990). The RAVLT consists of five learning trials with a 15-word list, one trial of a second 15-word list, followed by recall and recognition memory trials. Forty-seven patients in our sample were classified as mild, 32 as moderate

and 7 as severe. The elderly controls displayed a clear learning curve, good recall and recognition memory with minimal intrusions. The mild Alzheimer group displayed very little learning over trials, very poor recall and recognition memory with many intrusions on the recognition trial ($p<.0001$ for each group comparison, t-tests). Using a cutoff score of 2 SD below the control group mean, Trial V, Recall and Recognition minus intrusions were considerably better in discriminating Alzheimer patients from normal aging than Trials I - IV. Recall was the best at correctly identifying the elderly controls (100% controls; 68% mild, 91% moderate, 86% severe). Trial V was the best at correctly identifying DAT patients (96% controls; 79% mild, 100% moderate, 100% severe). Recognition memory minus intrusions correctly classified 94% of the controls, and 74% mild, 97% moderate, and 100% severe DAT. Using a criterion of any one of the three most sensitive subtests appreciably increases the percentage of correct classifications of mild DAT patients at the slight cost of 1 extra false positive error (92% controls, 87% mild, 100% moderate, 100% severe).

In summary, the ADAS, Semantic Word Fluency and the RAVLT each considered alone have considerable utility in the early detection of dementia of the Alzheimer type. Hit rates for patients with mild dementia of the Alzheimer type (MMSE $\geq$ 20) were 91% correct classifications using the cognitive portion of the ADAS, 85% using a semantic word fluency test, and 87% using a criterion of any one of the three most sensitive RAVLT subtest scores. Some combination of these three tests and the addition of other tests in our comprehensive screening battery (e.g., the Boston Naming Test) may increase the hit rate for mild patients even further.

## CONCLUSION

Although neuropsychological tests have the potential to reliably detect early dementia of the Alzheimer type, the currently published normative values are generally inadequate. The NINCDS-ADRDA task force (McKhann, et. al., 1985) operationally defined impairment in a domain of functioning as a score below the 5th percentile level compared to appropriate normative data controlling for age, gender and education. However, this task force also pointed out that there are no normative values for many of the tests which they recommended. The lack of precise normative data makes it very difficult to reliably judge whether a score is abnormal or not for a particular individual.

Normative studies are being currently conducted in a number of laboratories and those examiners who have access to this growing normative base should be better able to reliably detecting early dementia. A good normative base is essential to improving our ability to make a differential diagnosis of early Alzheimer dementia from normal aging and from other forms of abnormal aging. The clinical utility of different neuropsychological tests should be determined by calculating and publishing the "hit" rates for correctly classifying elderly controls and early Alzheimer patients in which severe impairment is operationaly defined a greater than 2 SD below the control group mean. Studies are also needed to determine which tests are

sensitive to the progressive worsening of the disorder, since tests which are most sensitive to early dementia are usually the least sensitive to monitoring change over time. Objective demonstration of progression of the disorder is very important in confirming a diagnosis of dementia of the Alzheimer type.

Future research should also determine the ability of neuropsychological tests to detect very early dementia of the Alzheimer type. It may be that mild to moderate impairment on one or more specific neuropsychological tests (i.e., between 1 and 2 SD below the mean of an appropriate control group) is an indication patients "at risk" or "at high risk" for dementia of the Alzheimer type. Some have proposed this predementia designation for patients with cognitive impairments which have not yet met the NINCDS criteria of severe impairments (> 2 SD below an appropriate control group). Longitudinal prospective studies need to determine the probability of developing Alzheimer disease in patient with and without mild-moderate cognitive impairments on sensitive neuropsychological tests.

There is evidence that cognitive impairment (as measured by sensitive psychometric tests) precedes impairments in functioning in everyday life (Grady, et. al., 1988; LaRue and Jarvik, 1987). This is probably especially true in the retired elderly who are less likely to be engaged in cognitive demanding tasks in job performance is being evaluated. Cerebral metabolic changes may, however, even precede the cognitive changes (Grady, et. al., 1988). According to the "threshold" model, neurological changes in the brains of Alzheimer patient, e.g., plaques, tangles, cell loss, cholinergic and other neurotransmitter deficits, must reach a certain critical level or threshold before performance on demanding psychometric tests or everyday functioning is affected (Grady, et. al., 1988). Thus, while sensitive neuropsychological tests have the potential of detecting early and maybe even very early Alzheimer's dementia, Alzheimer disease can be presumed to have began some time before it is actually manifested even in subtle cognitive impairment. Theoretically, biological markers should exist which precede even subtle cognitive impairment.

In summary, a neuropsychological evaluation of the cognitive functioning of a patient suspected of having Alzheimer's disease is necessary in order to objectively describe the severity, extent and pattern of the cognitive and memory impairments. A comprehensive description of the patient's cognitive impairments is important both to the differential diagnosis of the patient's disorder and to the care and treatment planning for the patient (McKhann et. al., 1984). It can provide a baseline against which future assessments can be compared. Comparison with a baseline is necessary in order to objectively measure the course of the disorder, to confirm the diagnosis and to evaluate treatments. There can be many different causes for the syndrome of dementia, including reversible causes, which underscores the necessity for an accurate differential diagnosis.

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# Part 3

# Neurochemistry



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# Diagnosis of Alzheimer Disease

J.P. Blass, R.S. Black, K.A. Nolan and A. Kurita
*Altschul Laboratory for Dementia Research*
*Cornell University Medical College at Burke Rehabilitation Center*
*White Plains, NY*

## INTRODUCTION

Diagnosis plays a critical role in the development of improved therapies. Diagnosis is a particularly important issue in therapeutic trials for disorders such as Alzheimer's disease, which are defined primarily by clinical and pathological criteria rather than by laboratory tests. In conducting a therapeutic trial, investigators must choose a group of "patients" believed to suffer from the disorder being studied. The investigators must often (although not invariably) choose a comparison group of "control" subjects believed not to be suffering from that disorder. For instance, normal controls are not needed in trials in which the effects of a putative therapy are compared to those of a placebo in a group of patients all of whom are classified as having the disorder for which the treatment is being tested, nor are comparison subjects needed in crossover trials in which each patient acts as his or her own control.

It is routine practice at the present time to establish strict inclusion and exclusion criteria for participation in a therapeutic trial before the study is begun. These criteria derive from the diagnostic formulations in use at the time the study is designed. As concepts change and as new technology reaches clinical application, diagnostic criteria can change; for instance, the wide availability of MRI scanning has altered the criteria for the diagnosis of vascular dementia (Seiderer et al, 1989; Kertesz et al, 1988; Council, 1988). For a chronic disorder such as Alzheimer's disease, the conduct of a rigorous clinical trial involving enough subjects to allow a confident conclusion, avoiding both type I and type II errors, typically requires several years. When a disorder is the subject of vigorous research leading to the rapid development of new concepts, as is currently true for Alzheimer's disease, there is a serious risk that inclusion and exclusion criteria for a rigorous therapeutic trial are likely to appear out of date by the time the trial is published, even though the criteria were chosen meticulously to reflect consensus views at the time the trial was designed. Furthermore, the results of a meticulously conducted and carefully analyzed therapeutic trial are likely to themselves generate new information which will raise new questions and modify diagnostic criteria for subsequent therapeutic trials in the disorder being studied. These considerations suggest that some discussion of general concepts of "diagnosis" should precede a discussion of diagnostic techniques in therapeutic trials for Alzheimer's disease and other dementias, since the diagnostic criteria and

techniques in use at the time this chapter is written are likely to change in the coming years.

"DIAGNOSIS" AS A CONCEPT

In the opening chapters of his monograph on The Role of Diagnosis in Psychiatry, Kendall (1975) points out that the term "diagnosis" can have various meanings depending on the context in which it is used. He traces this divergency to the ancient Greek physicians. The school of Cnos followed more Platonic approaches and looked on the manifestations in individual patients as more or less accurate expressions of an idealized disease entity which, because of its general validity, was more worthy of study than the particular manifestations of the illnesses in individual patients. The Platonic viewpoint persists in modern textbook descriptions of the "typical patient." The Hippocratic physicians of the school of Cos followed a more Aristotelian approach, concentrating on the accurate description of manifestations in individual patients or groups of patients and on the responses of the patients to therapy. This more mechanistic approach persists in descriptions of illnesses in terms of pathophysiological and pharmacological mechanisms. Kendall (1975) has pointed out the tension between these two viewpoints has persisted for over two millennia and has been productive for medicine. In the seventeenth century, Thomas Sydenham combined these approaches in an approach which was unorthodox in his day but has dominated the classification of illnesses since his time (Dewhurst, 1966). On the one hand, Sydenham was a meticulous empiric observer of patients and their response to therapy, accurately describing syndromes which are still important diagnostic entities. On the other hand, he felt that diseases, defined by clinical criteria and response to therapy, were specific entities which could be classified as species of plants were being classified by his contemporary Linnaeus. Among the triumphs of Sydenham's approach were his description of "quartan fever" and its response to quinine, centuries before malarial parasites were recognized.

Kendall (1975) also pointed out that although the terminology of specific diseases remains useful, the concept of diseases "like so many golden guineas waiting to be discovered in the sand" is theoretically flawed. Every human being is known to differ from every other human being, and the illness in each human being is known to differ in detail from that in all other human beings if examined and classified in enough detail, even hereditary disorders among identical twins (Nee et al, 1986). In this sense, no two human beings have exactly the same illness. On the other hand, terms like "stroke," "partial complex epilepsy," and "depression" efficiently communicate large bodies of information to trained health professionals who have learned and understand the nomenclature, even though each of these entities itself consists of clinically and therapeutically distinct subgroups, and the manifestations of these conditions vary significantly among the patients affected by any of them, even within the subgroups. Description of medical phenomena in terms of "diseases" is simply too useful to be discarded.

Specific "diseases" such as Alzheimer's disease are, from this viewpoint, convenient abstractions for describing groups of clinical phenomena and for focusing research on particular clinical problems. Diseases can be dropped from the nomenclature as new information reduces their utility, even if they can still be readily recognized by clinical criteria; examples include "pthisis" and "dropsy". Diseases can be redefined more precisely in terms of new knowledge: for instance, Tay-Sachs disease into $GM_2$

gangliosidosis. Diseases can be subdivided into clinically more useful entities; examples include the subtypes of diabetes or of neuropathies. And, of course, diseases can be newly recognized, such as AIDS.

A set of problems arise, however, when investigators move so far in the direction of the physicians of Cnos that they treat the diseases as described in textbooks or in consensus conferences as the real entities to be studied. Flippantly, the weakness of this approach is illustrated in the wisecrack that "the way to make a disease which is a public health problem disappear is to study it." When very strict criteria are applied, acceptable examples of even common diseases such as Alzheimer's disease can be hard to find. Levy et al (1984) pointed out that in therapeutic trials for Alzheimer's disease, it is typically necessary to screen 10 patients to find one acceptable for the trial. The problems of selection are exacerbated in studies in the geriatric age range, where the prevalence of coexistent disorders ("multiple morbidity") is high. These coexistent disorders can both cloud the clarity of the diagnosis of Alzheimer's disease and exclude patients from a pharmacological trial because their concurrent illnesses put them at increased risk from the side effects of the medication being tested.

The problem of selection also exists in younger patients whose "disease" is defined by biochemical or other laboratory criteria. For instance, in a study to determine the relation between cholesterol levels and coronary heart disease, 480,000 men were screened to find 3,806 (0.8% of the sample) who had type II hyperlipoproteinemia but no clinical evidence of coronary heart disease (Lipid Research Clinics Program, 1984a,b; Texon, 1989). Careful statistical analysis allowed the investigators who carried out this study to conclude that the treatment they were testing (cholestyramine) was effective in the group of individuals studied, although at the end of the seven year study the total number of excess deaths in the 1,900 individuals who received placebo compared to the 1,906 who received cholestyramine treatment was 3. A concern must, however, remain as to whether the results observed in less than 1% of the individuals screened can be applied to the whole population. The pathophysiological purity of the group studied is theoretically attractive. The extrapolation of the results in this highly selected group to the larger group remains, however, an unproven even if attractive hypothesis until tested scientifically in a rigorous trial of an adequately large clinical sample of the larger, pathophysiologically impure group.

Modern medical science and technology allow extremely refined, precise, and narrow descriptions of clinical entities, now including Alzheimer's disease (Small et al, 1989; Gusella, 1989; Henderson et al, 1989; Fitch et all, 1988; Hyman et al, 1988; Tierney et al, 1988; Becker et al, 1988; Jagust et al, 1988; Zubenko et al, 1987a,b,c; Moss et al, 1986; Bondareff & Mountjoy, 1986; Khachaturian, 1985a,b). Although the possibility has been raised that patients with familial Alzheimer's disease will be grouped according to the gene or chromosome affected, molecular genetic findings in Alzheimer's disease remain controversial (Fitch et al, 1988; Gusella, 1989). However, very detailed subdivisions of Alzheimer's disease can now be based on neuropsychological (Henderson et al, 1989; Becker et al, 1988; Berg et al, 1987; Moss et al, 1986), PET scan (Small et al, 1989; Jagust et al, 1988), or pathoanatomical criteria (Tierney et al, 1988; Hyman et al, 1988; Bondareff & Mountjoy, 1986; Khachaturian, 1985a). It is increasingly possible to describe biochemical subgroups during life - for instance, patients with and without increased platelet membrane fluidity (Zubenko et al, 1987a,b,c). It is likely that investigation will add to the armamentarium of laboratory

tests which can be used to define subgroups of patients with Alzheimer's disease. As yet, however, these subdivisions do not correlate well with age of onset, rate of progression, or other characteristics of the clinical course.

The choice of appropriate diagnostic inclusion and exclusion criteria for therapeutic trials in Alzheimer's disease, as in other diseases, will continue to require judgement. The investigators will need to strike a balance between conflicting demands. On the one hand, the criteria should be precise enough to define a reasonably homogeneous group of patients in whom pathophysiologically and therapeutically precise questions can be addressed and even small effects detected with confidence. On the other hand, if the criteria are too strict, they will allow conclusions only about a limited population rather than about the bulk of individuals classified as having "Alzheimer's disease." The development of laboratory tests as aids to diagnosis is likely to increase, not decrease, the degree of judgement which investigators designing therapeutic trials must exercise. Sydenham provided a guideline when he emphasized that a "disease" is a meaningful entity when it can be defined by clinically available techniques and when its recognition allows the clinician to provide more effective treatment or more precise advice to the afflicted persons and their families (Dewhurst, 1966). In practice, the definition of inclusion and exclusion criteria requires judgement and is one of the most important parts of the design of a therapeutic trial.

## CURRENT DIAGNOSIS OF ALZHEIMER'S DISEASE

The diagnosis of Alzheimer's disease, in both clinical practice and for therapeutic trials, was considered to be a major problem in the late 1970s and early 1980s. Consensus criteria have, however, been developed for the diagnosis both clinically (McKhann et al, 1984) and neuropathologically (Khachaturian, 1985) diagnosis. Prospective studies have shown correlations between the clinical and pathological findings using these criteria of between 64% and 100% (see discussion below). At the present time, these sets of criteria are in conventional use in the United States, and patients in therapeutic trials for Alzheimer's disease in this country usually meet NINCDS-ADRDA criteria for "probable Alzheimer's disease (McKhann et al, 1984; and see below). A similar but not identical set of criteria have been developed by the Medical Research Council in Great Britain (Anonymous, 1989). A number of problems remain, however, related not only to the specificity of diagnosis but also to unsettled issues in the pathophysiology of the disorder.

### Consensus Clinical Criteria

The most widely used clinical criteria in the United States are those developed in a consensus process sponsored by the National Institute of Neurological Disorders (then the NINCDS) in collaboration with the Alzheimer's Association (then the ADRDA) and published in 1984 (McKhann et al, 1984). The first step in applying these criteria is to determine whether or not the subject has the dementia syndrome, defined as global cognitive impairment in a clear sensorium. While the consensus conference did not list a minimum duration for the cognitive impairment, our experience suggests that it is risky to make the diagnosis of a dementing syndrome unless cognitive disabilities have lasted for at least 3 and better for at least 6 months. Global cognitive impairment is defined as deficits in at least 2 or more areas of cognition, normally including memory; the areas of cognition listed by the consensus conference were memory, orientation, language skills,

praxis, attention, visual perception, problem solving skills, and social function. As noted below, major deficits in attention raise the question of delirium or a coexisting delirium. Global cognitive impairment can be readily documented by a variety of brief, formalized neuropsychological procedures (Knopman et al, 1989; Berg et al, 1987; Fillenbaum et al, 1987; Khachaturian, 1985b; Anthony et al, 1982). Perhaps the most widely accepted of these procedures in the United States at the present time is the Mini-mental Status exam of Folstein, Folstein and McHugh (Anthony et al, 1982). A standardized battery of neuropsychological tests for patients with possible Alzheimer's disease has been introduced by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD; Morris et al, 1989). A more detailed discussion of the extensive literature on the neuropsychological evaluation of patients with Alzheimer's disease and other dementias is outside the scope of this chapter (Khachaturian, 1985b).

A critical element in the diagnosis of dementia which requires clinical judgement is determining whether or not the patient has a clear sensorium, ie whether or not the patient has a delirium rather than or in addition to a dementia. Engel and Romano (1959) pointed out that while both delirium and dementia cause global cognitive deficits, dementia is characteristically associated with irreversible, anatomic damage to the brain while delirium is typically associated with potentially reversible impairments of brain cell functions (eg drug effects, metabolic encephalopathies). Delirium can, of course, be superimposed on an underlying dementia. Clinically, patients with Alzheimer's disease or other dementias typically pay attention to the examiner, at least in the earlier stages of the disorder, even when they do not understand what the examiner is saying. They typically have a more stable, slowly progressive deficit, although fluctuations can be seen associated with emotional stress, medication, intercurrent illnesses, or other stressors. Typically, the attention of patients with delirium wanders, and their deficits fluctuate. The distinction is usually relatively straightforward but can be very difficult. A particular source of confusion is the chronic use of medications which can interfere with cognition. The list of these is long, and includes both prescription and non-prescription drugs including drugs of abuse (Blass & Plum, 1983). It is most important to have the caretaker bring in a list of <u>all</u> the medications to which the patient has recently been exposed, if possible by bringing in to the examiner all the medications in the patient's home. It is also important to remember that the brains of older individuals can be particularly sensitive to the effects on cognition of even such common medications as antihistamines and digitalis. Individuals with early and otherwise clinically unimportant Alzheimer's disease or other dementing disorder can be "tipped-over" into frank clinical dementia by a dose of a medication which would have negligible effects on the cognition of a younger and healthier person. Despite these problems, however, prospective series indicate that a careful clinical examination can identify the dementia syndrome accurately in over 95% of cases (Forette et al, 1989; Reding et al, 1984). Nevertheless, the concomitant or recent use of drugs which can impair cognition is now among the criteria for exclusion from most therapeutic trials for Alzheimer's disease. The choice of the list of drugs which exclude patients can be a difficult problem. The more medication-free the patients, the "cleaner" the group being studied. The frequent multiple morbidity in patients in the age range in which Alzheimer's disease is most common implies that many of them will be taking digitalis, antihypertensives, or other medications with potential effects on the nervous system. Choosing too extensive a list of excluding medications not only

makes recruitment more difficult, time consuming, and expensive, but also raises the confound of excessive selection.

In a patient with the dementia syndrome, the diagnosis of Alzheimer's disease is a diagnosis of exclusion. Alzheimer's disease is confidently diagnosed in individuals who have a course consistent with the disease, namely an insidious onset and a more or less steady downhill course, and in whom careful clinical examination reveals no other cause adequate to explain the dementing illness. The NINCDS-ADRDA criteria recognize three levels of confidence in the diagnosis (McKhann et al, 1984). Possible Alzheimer's disease is diagnosed in individuals who have a course and clinical characteristics consistent with the disease but have other clinical problems which may themselves interfere with cognition such as depression or a coexistent metabolic disorder. Patients who have the dementia syndrome, a course consistent with Alzheimer's disease, and no confounding medical or psychiatric problems are classified as having probable Alzheimer's disease; most subjects in therapeutic trials now fall into this category. Definite Alzheimer's disease requires histopathological confirmation, at either biopsy or autopsy. In clinical research on Alzheimer's disease including therapeutic trials, it is now conventional to perform an extensive evaluation including CT or MRI scans, as recommended by the NINCDS-ADRDA consensus panel (McKhann et al, 1984), to rule out other causes of dementia or a confounding chronic delirium. It should be noted, however, that a careful prospective study of 200 patients led to the conclusion that in ordinary clinical practice, the diagnosis can be made with high accuracy with a much less extensive evaluation, particularly in patients well known to the physician (Larson et al, 1986). The differential diagnosis of dementia in the elderly is beyond the scope of this chapter, but has been extensively reviewed (Ettlin et al, 1989; Foley et al, 1988; Cummings & Benson, 1984; Blass & Plum, 1983). Three issues which do, however, deserve special mention are the recognition of early Alzheimer's disease, the relationship between depression and dementia, and the relationship between between circulatory and degenerative dementias (eg multi-infarct dementia and Alzheimer's disease).

Since Alzheimer's disease leads to progressive anatomical damage to the brain including irreversible loss of neurons, there would be an obvious advantage to diagnosing the disorder in its earliest stages where therapy might have the chance to be most effective. Recent studies indicate that performance which is below age norms but not low enough to meet conventional criteria for dementia identifies a population at high risk for developing a progressive dementing disorder, although they do not distinguish Alzheimer's disease from other dementias (Katzman et al, 1989; Rubin et al, 1989). Katzman et al (1989) followed 434 individuals originally aged 75-85 for 5 years, and found that the incidence of dementia was 20-fold higher in those who originally made 5-8 errors on a 33 question mental status examination compared to those who made only 0-2 errors (0.6%/year compared to 12%/year). It is important to note, however, that even among those making 5-8 errors, 40% did not develop dementia during the time period of the study. Rubin et al (1989) using the Clinical Dementia Rating scale found that 11/16 patients who scored below age norms went on to develop clinical dementia or met pathological criteria for Alzheimer's disease at autopsy. Grober et al (1988) have suggested the use of cued recall to detect early dementia, and Knopman (1989) the use of delayed word recall. Morris and Fulling (1988) have pointed out that deterioration on standard tests over a period of months is a good predictor of early dementia. Attempts to develop sensitive and relatively simple tests to distinguish early Alzheimer's disease from other

dementias continue. Storandt et al (1989) concluded, however, that there are as yet no neuropsychological techniques to allow the confident early diagnosis of Alzheimer's disease. Their conclusion agrees with that of Berg et al (1987). Nor, as noted below, are validated laboratory tests to aid early diagnosis available as yet.

Possible confusion between cognitive impairment due to depression and that from dementia has received extensive study (Ettlin et al, 1989; Merriam, 1988; Bleeker et al, 1988; Cummings et al, 1987; Shalat et al, 1987; Reding et al, 1984, 1985; Cummings & Benson, 1984; Kral, 1983; 48-52). The relationship is complex. Depression can impair performance on psychological testing, particularly under time-limited or other potentially stressful circumstances. However, a high percentage of elderly patients who develop depression without clear cut dementia by neuropsychological criteria at the time of initial clinical examination go on to develop a frank dementing disorder. The incidence of dementia in elderly patients who present with depression varies with the time of follow-up. It was 50% at three year follow-up in the series of Reding et al (1985), but over 90% (20/22 patients) after follow-up of up to 18 years in the series of Kral (1983). Furthermore, the symptoms of depression are common in patients with Alzheimer's disease, being reported in over 86% of the patients examined by Merriam et al (1988). Others have found lower but still significant incidence of depressive symptomatology - for instance 50% by Cummings et al (1987). Depressed patients who score below age-norms on simple and robust neuropsychological tests such as the Mini-Mental Status Examination are likely to have a dementing illness as well (Bleeker et al, 1988; Reding et al, 1985). In therapeutic trials in Alzheimer's disease, it is now conventional to exclude patients who are or have recently been on antidepressant medication or who have a history of major depressive disorder, to avoid the confound of depression.

The second most common cause of the dementia syndrome in the United States, after Alzheimer's disease, is disease due to circulatory factors (Wade et al, 1987; Larson et al, 1986; Cummings & Benson, 1984; Reding et al, 1984). The presence of Alzheimer's disease does not prevent the development of concomitant cerebrovascular damage, and cerebrovascular disease does not prevent the development of Alzheimer's disease, so that a significant proportion of patients - approximately 10-20% in most series (Wade et al, 1987; Reding et al, 1984,1985) have both. Although the term "multi-infarct dementia" is widely used to refer to dementia due to cardiovascular factors, circulatory disease can lead to brain damage by a number of mechanisms other than multiple small infarcts - for instance, by hypoxic-ischemic brain damage due to cardiac arrest (Blass, 1987). While it is clear that Alzheimer's disease is not due to atherosclerosis or other damage to the large vessels of the brain, the contribution of small vessel disease and specifically of cerebrovascular amyloidosis is unsettled (Joachim et al, 1988; DelaCourte et al, 1987; Mandybur, 1975). Hachinski introduced an ischemic scale to quantify the likelihood that dementia was due to vascular factors, and prospective studies have indicated that this scale detects patients in whom vascular disease contributes to their dementia with an accuracy of over 80% (Wade et al, 1987; Rosen et al, 1980). Currently, in therapeutic trials for Alzheimer's disease, an unmodified Hachinski ischemic score of $\geq 4$ is usually one of the exclusion criteria. Radiological criteria for excluding cerebrovascular damage are discussed below.

Consensus Neuropathological Criteria.

Khachaturian (1985a) has described the results of a consensus conference on the neuropathological criteria for Alzheimer's disease. Subsequently a standard protocol for neuropathological examination has been developed by the CERAD group, as well as a standardized semi-quantitative format for reporting the results (unpublished; cf Morris et al, 1989). The consensus requirements for the neuropathological diagnosis are not, of course, meant to preclude more extensive examination of the brain at autopsy. A more complete discussion of the neuropathological diagnosis and of the use of immunocytochemical and other newer reagents to aid in that diagnosis, including the Alz-50 monoclonal antibody (Love et al, 1988; Tabaton et al, 1988), is beyond the scope of this chapter.

Concordance of Clinical and Neuropathological Diagnoses

When consensus clinical criteria (McKhann et al, 1984) have been used in prospective studies to predict which patients will meet consensus neuropathological criteria (Khachaturian, 1985a) for Alzheimer's disease, the concordance between clinical and pathological criteria has varied from 100% to less than 65% (Boller et al, 1989; Tierney et al, 1988; Morris et al, 1989; Joachim et al, 1988; Huff et al, 1987; Martin et al, 1987). Martin et al (1987) found concordance in 11/11 biopsied patients, and Morris et al (1989) in 37/37 patients who came to autopsy. In contrast, Boller et al (1989) reported that only 39/57 patients carefully diagnosed as having Alzheimer's disease in the Alzheimer's disease center at the University of Pittsburgh met neuropathological criteria. Joachim et al (1988) reported that 87% of the brains submitted to McClean Hospital in Massachusestts as "Alzheimer's disease" met neuropathological criteria. Tierney et al (1988) concluded in their series that depending on the neuropathological criteria applied, the clinicopathological agreement ranged from 64% to 86%. Probably concordance also depends on how tightly the clinical diagnosis of Alzheimer's disease is defined.

An important caveat about the use of neuropathology as the "gold standard" for diagnosis is that patients can have the requisite number and distribution of the diagnostic plaques and tangles at autopsy without having been demented during life (Katzman et al, 1988; Crystal et al, 1988; Fuld et al, 1987; Kokmen et al, 1987; Ullrich, 1985). It has been proposed that these individuals may have had a greater reserve of brain to begin with (Crystal et al, 1988), and that they may have "preclinical" Alzheimer's disease (Fuld et al, 1987). Nevertheless, since the number of individuals who do not have clinically significant Alzheimer's disease is 5 to 6-fold the number who do have the disorder, even among people in their 80s (Kokmen, 1987), the existence of the characteristic neuropathological lesions in even a relatively small fraction of the normal population decreases the value of these lesions as a diagnostic test. Studies to date do not allow the confident calculation of a false positive rate for the diagnosis of dementia accompanying Alzheimer lesions at pathology, but suggest that the false positive rate in a prospective study would be substantial (Katzman et al, 1988; Crystal et al, 1988; Kokmen et al, 1987). The corollary of this suggestion is that any diagnostic test based on detecting the lesions or the materials which accumulate in the lesions is likely to show a significant rate of false positives. Whether or not that false-positive rate is acceptable for a laboratory adjunct to diagnosis depends on the purposes for which the laboratory test is used.

Consensus criteria for "probable Alzheimer's disease" are widely used to define a population homogeneous enough for therapeutic trials, but the need for more precise laboratory or other diagnostic instruments as adjuncts to diagnosis is also widely accepted.

LABORATORY AIDS TO DIAGNOSIS

At the present time, the major aid that laboratory testing provides for the diagnosis of Alzheimer's disease is in excluding other potential causes of dementia (McKhann et al, 1984). Among the possibilities for laboratory adjuncts to diagnosis are biopsy, neuropsychology, radiology, and the study of relatively available non-neural tissues.

Brain biopsy is a highly invasive technique which has not seen wide-spread use (Martin et al, 1987). It is, however, relatively safe and is available if circumstances were to warrant: for instance, if a treatment became available which appeared effective but had potential side effects more serious than those of brain biopsy.

Neuropsychological techniques are discussed above, particularly in relation to early diagnosis. They have the advantage of being non-interventive and of measuring directly the disabilities of major clinical interest. Neuropsychological techniques currently available have not, however, been demonstrated to distinguish reliably between vascular and Alzheimer dementias. Furthermore, the interpretation of many of the test scores is limited by relatively little knowledge of the effects of education, ethnicity, and normal (normative) aging.

Radiological techniques are already in wide-spread use. Most patients in the United States now have a CT scan or an MRI scan before the diagnosis of Alzheimer's disease or other specific dementing illness is made. The most important contribution of radiological examinations to the diagnosis of Alzheimer's disease up to now has been to rule out the presence of other lesions, such as tumors or subdural hematoma, which can lead to the dementia syndrome. The brains of patients who have Alzheimer's disease typically show atrophy (Riege & Mettler, 1988; Johnson et al, 1987). In patients below the age of 65 enlargement of sulci and ventricles can be a useful confirmatory finding, but in patients in the older age range atrophy is not enough more marked in Alzheimer patients than in age-matched controls to be a useful discriminant (Riege & Mettler, 1988; Johnson et al, 1987). Progressive atrophy on successive CT scans has, however, been reported to distinguish Alzheimer patients from cognitively intact individuals even in the older age range (Luxemburg et al, 1987). A recent study proposes that hippocampal atrophy detected with coronal (reconstructed) CT images can be a valuable sign (de Leon et al, 1989). Both CT and MRI scanning are now widely used to evaluate the presence of cerebrovascular disease and particularly white-matter disease. On MRI, "unidentified bright objects" (UBOs) and periventricular lucencies are seen in approximately 5-25% of cognitively intact elderly subjects, approximately 30% of patients with Alzheimer's disease, and over 70% of patients with circulatory ("multi-infarct") dementia (Hunt et al, 1989; Seiderer, 1989; Kertesz, 1988; Council, 1988; Aharon-Peretz et al, 1988; Gupta et al, 1988; Rezek et al, 1987; Luxemburg et al, 1987; Johnson et al, 1987). On CT scan, abnormalities of "leukoaraiosis" have been reported in as many as 30/31 patients with circulatory dementia and 9/18 with Alzheimer's disease (Luxemburg et al, 1987). The clinical significance of white matter abnormalities on MRI (Hunt et al, 1989) and CT scans (Steingart et al, 1987) and their neuropathological correlates remain controversial.

White matter abnormalities on MRI or CT scan have, however, been reported to correlate with Binswanger's disease, which can itself cause the dementia syndrome (Gupta et al, 1988; Rezek et al, 1987; Brun & Englund, 1986). Furthermore, recent neuropathological studies suggest that axonal degeneration and white matter damage occur in over 60% of patients who meet clinical and neuropathological criteria for Alzheimer's disease and may even be an intrinsic part of the Alzheimer's process (de la Monte, 1989; Brun & Englund, 1986) . Although disease of the large cereberal vessels does not correlate with the occurrence of Alzheimer's disease (Mandybur, 1975), recent evidence that plaque and vascular amyloid has the same composition has led to renewed interest in the role of amyloid angiopathy in Alzheimer's disease (Glenner, 1988). The presence in an as yet undetermined portion of cognitively intact individuals of enough plaques and tangles to make the neuropathological diagnosis of Alzheimer's disease suggests that if a radiological procedure to detect plaques and tangles were discovered, it might prove as "nonspecific" as the radiological changes associated with cerebrovascular disease. At the present time, evidence for cerebrovascular disease on CT or MRI scan is often used as an exclusion criteria for therapeutic trials in Alzheimer's disease and for an inclusion criterion for trials of circulatory ("multi-infarct") dementia, but the scientific basis for doing so has not been well established.

Procedures which measure cerebral blood flow or metabolism remain research techniques (Small et al, 1989; Jagust et al, 1988; Reege & Mettler, 1988), although newer modifications may increase their diagnostic value (Prohovnik et al, 1988).

Clinical Laboratory Studies are now routinely a part of diagnostic evaluation of Alzheimer's disease, to rule out metabolic or nutritional disorders which can lead to or exacerbate cognitive impairment (Larson et al, 1986; McKhann et al, 1984). Attempts to develop positive diagnostic tests for Alzheimer's disease have utilized the detection of abnormalities in tissues more readily available than brain (Blass et al, 1985,1989). They are broadly of two types: attempts to identify cellular or molecular abnormalities, and immunochemical techniques to detect the epitopes associated with the materials in the plaques and tangles.

Examples of the first type of investigation are the as yet controversial attempts to identify a genetic linkage marker (RFLP); Gusella (1989) has described the prospect of confidently identifying a gene for Alzheimer's disease in the currently available kindreds as "daunting." Increased RBC/plasma choline (Blass et al, 1985) and increased platelet fluidity (Zubenko et al, 1987a,b,c) are found in subgroups of patients with depression as well as in subgroups of patients with Alzheimer's disease and are not likely to be the basis of diagnostically useful laboratory tests. A number of other abnormalities described in non-neural tissues have not yet been tested for their diagnostic value, including abnormal cellular calcium homeostasis, described in cultured skin fibroblasts (Peterson et al, 1985) and lymphocytes (Gibson et al, 1987) or abnormalities of protein kinase C in fibroblasts (Hunyh et al, 1989).

Immunochemical studies have examined spinal fluid for the presence of materials reacting with the Alz-50 monoclonal antibody (Hyman et al, 1988), which appears to recognize an abnormal 68 kDa protein and tau proteins on denaturing gels (Love et al, 1988; Kseziak-Reding et al, 1988) and antibody 5-25 which recognizes an epitope on ubiquitin which is incorporated into paired helical filaments (Perry et al, 1989). An antibody to the Alzheimer

precursor protein (APP) has been reported to react immunocytochemically more often with skin of Alzheimer than of control subjects, although the 15% frequency of reactivity in control subjects suggests that a laboratory test based on this reactivity may be of limited diagnostic value (Joachim et al, 1989). We have found that a significantly higher proportion of fibroblasts from Alzheimer patients than from control subjects react with several antibodies to paired helical filaments, when the fibroblasts are cultured in media modeled on those used to grow cultured neurons from fetal brain (Blass et al, 1989). There was no overlap in 20 patients compared to 20 control subjects, and studies are now underway to identify the materials responsible for the immunocytochemical reactivity. It should, however, be emphasized that the materials which react with antibodies to the abnormal materials which accumulate in Alzheimer's disease also accumulate in a number of other degenerative dementing disorders of the elderly, including Pick's disease, supranuclear palsy, and Parkinson's disease with dementia (Love et al, 1988; Tabaton et al, 1988; Galloway et al, 1988). The specificity of the immunochemical reactivity in the periphery seems intuitively unlikely to be greater than the specificity of the reactivity in the brain.

As discussed in the opening section of this chapter, precedents in the application of clinical laboratory tests to other diseases suggest that no single laboratory test will correlate perfectly with the clinico-pathological correlation as currently defined. They may, however, allow the clinical diagnosis to be made with greater confidence. They may also help to define meaningful subgroups including perhaps a population who will be accepted to constitute the "core" group of Alzheimer's disease.

## THERAPEUTIC INVESTIGATIONS AS A GUIDE TO DIAGNOSIS

The refinement of diagnostic concepts is a dialectic process to which carefully designed therapeutic trials can contribute. As a disease (thesis) is studied, new information is gathered which often leads to alterations in the original concepts (antithesis), eventually leading to a refinement or redefinition of the original diagnostic category (synthesis). Studies are then undertaken to improve knowledge of the newly redefined entity, which becomes the new thesis. In this process, the discovery of more effective therapeutic modalities can not only benefit the suffering patients and their families but lead to new insights into the biology of the disease being studied. Response or lack of response to a particular therapy can itself be a marker for a clinically significant category of patients, although the confident acceptance of such a category normally requires that other clinical or laboratory criteria be discovered by which that group of patients can be identified with reasonable statistical accuracy.

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# Markers of Cholinergic Dysfunction in Alzheimer Disease

Ezio Giacobini and Kiminobu Sugaya
*Department of Pharmacology, S.I.U. School of Medicine* and
*Regional Alzheimer Disease Assistance Center, Springfield, IL*

Rodger J. Elble
*Department of Medicine, S.I.U. School of Medicine* and
*Regional Alzheimer Disease Assistance Center, Springfield, IL*

## INTRODUCTION

Several studies suggest that impaired cholinergic function plays an important role in the memory and cognitive deficits of normal aging (Bartus et al., 1982). Numerous neurochemical studies performed on autopsy and biopsy samples of brain regions demonstrate a far greater involvement of the cholinergic system in Alzheimer disease (AD) (Giacobini, 1988). The cholinergic systems most affected by AD are summarized in Table I. The forebrain nuclei (mainly the basal nucleus), neocortical regions, hippocampus, ventral striatum, and amygdala are characteristically affected by this disease.

TABLE I
Cholinergic Pathology of Alzheimer Disease

| | | Cholinergic Representation | |
|---|---|---|---|
| | | Neurons | Projections |
| 1. | Neocortex (front. par. temp) | + | ++ |
| 2. | Hippocampus | - | +++ |
| 3. | Amygdala | - | ++ |
| 4. | Ventral striatum | ++ | - |
| 5. | Basal forebrain | +++ | - |
| 6. | Locus coeruleus | - | + |
| 7. | Raphe' | - | + |

Variable involvement of the cholinergic system is also seen in other dementing diseases such as Parkinson, Huntington and Pick disease (Table II). Toxic-metabolic encephalopathies in elderly patients frequently entail cholinergic dysfunction (Blass and Plum, 1983). Elders are particularly

susceptible to the adverse effects of widely prescribed anticholinergic drugs (Blazer et al., 1983; Sunderland et al., 1987) and are prone to the development of drug-induced delirium (Lipowski, 1989).

TABLE II
Involvement of the Central Cholinergic System in Cerebral Diseases or Syndromes

| | | | |
|---|---|---|---|
| A. | Degenerative Diseases<br>Alzheimer<br>Parkinson<br>Pick | B. | Metabolic encephalopathies<br>Korsakoff |
| C. | Delirium (acute confusional states) in Elderly Patients<br>anticholinergic agents<br>toxic-metabolic encephalopathies | | |

The presence of cholinergic dysfunction in such a wide spectrum of CNS diseases has prompted many investigators to ask whether acetylcholine (ACh) metabolism in brain is reflected by changes in cholinergic indexes that can be monitored through neural and extraneural markers. In a previous paper, we have summarized CSF studies with regard to ACh, ACh-related enzymes, and choline (Ch) (Giacobini, 1986). In this paper we will review cholinergic markers of AD in cerebral and extracerebral compartments. The review focusses on results from our laboratory.

TABLE III
Cholinergic Responses to Pharmacological Challenges in AD Patients

| | |
|---|---|
| 1. | If cholinergic blockade (scopolamine) can mimic the normal aging process, will patients display greater sensitivity? |
| 2. | Will patients be hyporesponsive to cholinergic stimulation (nicotine, arecoline)? |
| 3. | Will scopolamine hypersensitivity be predictive of progressive deterioration and sensitivity? |
| 4. | Will pharmacological challenges distinguish AD from depression (pseudo-dementia)? |

## CHOLINERGIC MARKERS IN ALZHEIMER DISEASE

### 1. Cognitive Function and Experimental Pharmacology

The administration of cholinergic receptor antagonists (antimuscarinics) to normal human controls (Drachman, 1974, 1978, 1983; Sitaram et al., 1978) and laboratory primates (Bartus, 1978) produces short-term dose-related memory impairment that mimics the memory dysfunction in AD. Elders, in particular, are sensitive to this effect of anticholinergic drugs (Blazer et al., 1983; Sunderland et al., 1985, 1987). Based on these clinical and experimental data, the issue of sensitivity to cholinergic agents in patients with major cholinergic deficits raises the questions of Table III. In particular, would cognitive and electrophysiological measures reflect dose-dependent dysfunction triggered by

anticholinergics in patients with AD? Could such tests serve as measures of central cholinergic impairment and prove useful in diagnosis and staging of AD?

Beatty et al. (1986) failed to obtain an effect of scopolamine in a small number of volunteers while Wesnes et al. (1987) confirmed that cholinergic blockade disrupts memory. Miller et al. (1988) demonstrated that a low dose of scopolamine (5 μg/kg i.m.) caused mild but measurable cognitive impairment in 18 psychiatrically healthy elderly subjects. Mohs and Davis (1985) and Sunderland et al. (1985, 1987) used scopolamine as a pharmacological probe of cholinergic mechanisms and found that 0.1-0.5 mg s.c. or i.v. impaired memory. In the Sunderland et al. (1985) study, cognitive testing revealed dose-dependent impairment in tests of vigilance, new learning, and knowledge memory. In our studies of 16 patients with AD (Elble et al., 1988), scopolamine infusion (0.161 $mg/m^2$ i.v.) impaired memory by an average of 9.0 ± 11.8% ($p < 0.05$). This effect

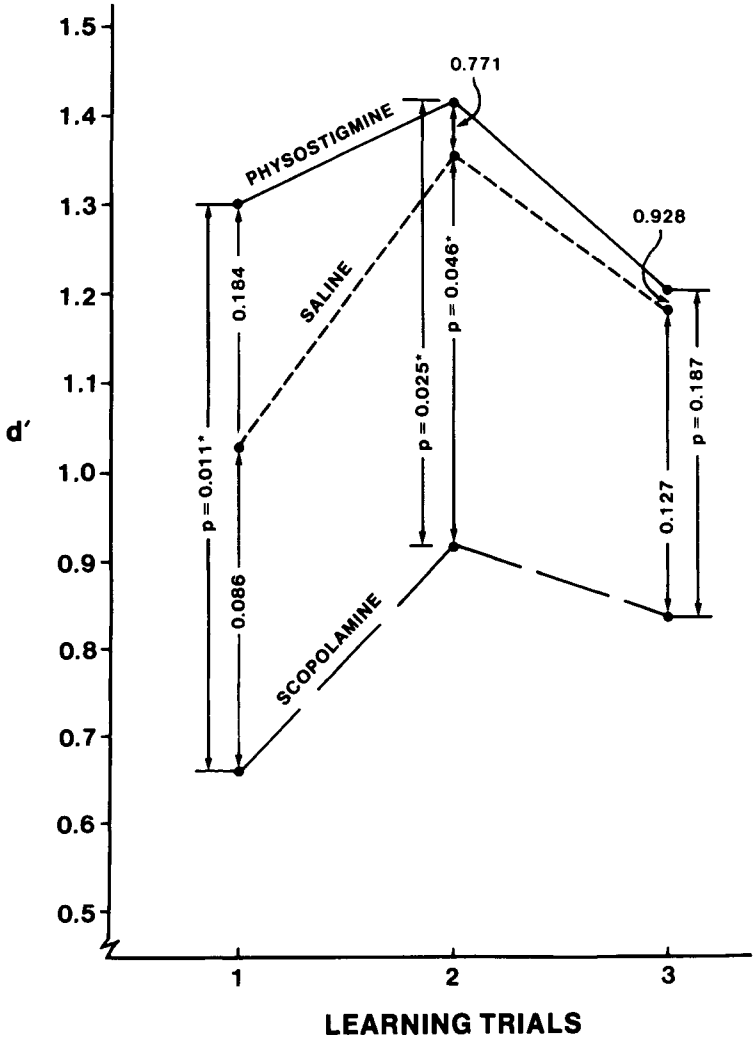



Figure 1: A trial-by-trial graph of memory performance following infusions of physostigmine, saline and scopolamine. The parameter d' was computed using the methods of signal detection analysis (Mohs and Davis, 1982) and is a measure of the information stored in memory about the sequence of words or pictures. The memory parameter d' was significantly reduced by scopolamine in trial 2 ($p = 0.046$). Physostigmine had no significant effect on d', but for all three memory trials, there was a consistent trend for increased d' with physostigmine and for reduced d' with scopolamine (from Elble et al., 1988).

was measured by using a word or picture recognition task in three consecutive placebo-controlled learning trials. Furthermore, physostigmine (Phy) and scopolamine had opposite effects on the information stored in memory during each of the three trials (Fig. 1). However, the response to scopolamine was not a predictor of Phy response and scopolamine-induced memory impairment was not correlated with Mini-Mental State or age.

In a recent series of pharmacological challenges in 83 neuropsychiatric patients and controls, Sunderland et al. (1988) contrasted the effect of scopolamine (0.1-0.5 mg i.v.) with infusion of two cholinergic agonists [arecoline (1-4 mg/hr) and nicotine (0.125-0.50 μg/kg/min)]. Alzheimer patients exhibited greater sensitivity to central cholinergic blockade than age-matched controls or elderly depressives. A differential sensitivity to arecoline and nicotine was also observed. Newhouse et al. (1988) also compared patients with AD to elderly depressed patients and found a greater sensitivity to scopolamine in AD. However, the sensitivity and selectivity of these pharmacologic probes are still unestablished, and the effect of these probes on specific neuropsychological measures may be expected to vary among patients with the same disease (e.g. AD) and particularly among patients with different diseases.

Regional cerebral blood flow (rCBF) measurements (Honer et al., 1988) and direct visualization of cholinergic receptors with PET scanning during scopolamine infusion could result in non-invasive tests with better diagnostic and prognostic accuracy.

2. Neuropsychological Function and EEG

Several studies have reported slowing of EEG pattern (delta activity) in AD and have tried to correlate EEG measures with the degree of dementia. The principal conclusions of these studies are listed in Table IV. Soininen et al. (1988) and Helkala et al. (1988), in a one year follow up study of 92 AD patients, found that slow wave activity was related to variation in specific neuropsychological functions. The slowing of EEG in AD patients has been suggested to correlate with progressive cholinergic denervation of the cortex (Neufeld et. al., 1988). In experimental animals, slow activity in neocortex has been associated with damage to the basal forebrain which is the primary source of cholinergic cortical afferents (Stewart et al., 1984). In particular, Buzsaki et al. (1982) have stressed the role of nucleus basalis and thalamus in the control of neocortical activity and suppression of delta activity. Buzsaki et al. (1988) recorded electrical activity in the neocortex of freely moving rats and found that increased neuronal firing of the nucleus basalis provides a background of cortical activity that may enhance the effect of other afferents to the neocortex. Application of antimuscarinic drugs such as scopolamine results in the abolishment of low-voltage fast activity and the appearance of delta waves during specific behaviors (Vanderwolf et al., 1981) (Table IV). Thus, both clinical (Helkala et al., 1988) and experimental (Buzsaki et al.,

TABLE IV

Experimental Cholinergic Deficits

| | |
|---|---|
| 1. | Muscarinic antagonists disrupt cognitive function in rodents and primates. |
| 2. | Treatment with muscarinic antagonists abolishes low voltage <u>fast</u> activity in EEG and generates <u>delta</u> waves. |
| 3. | Lesions of the basal forebrain deprive cortex of cholinergic input and produce increased slow wave activity in the EEG. |

1988) results suggest that changes in delta activity in AD patients may be related to cortical cholinergic dysfunction as reflected by deficits in retrieval from semantic memory (Table V). The utility of spectral EEG analysis in diagnosis and staging remains to be proven by longitudinal studies of the type of Soininen et al. (1988).

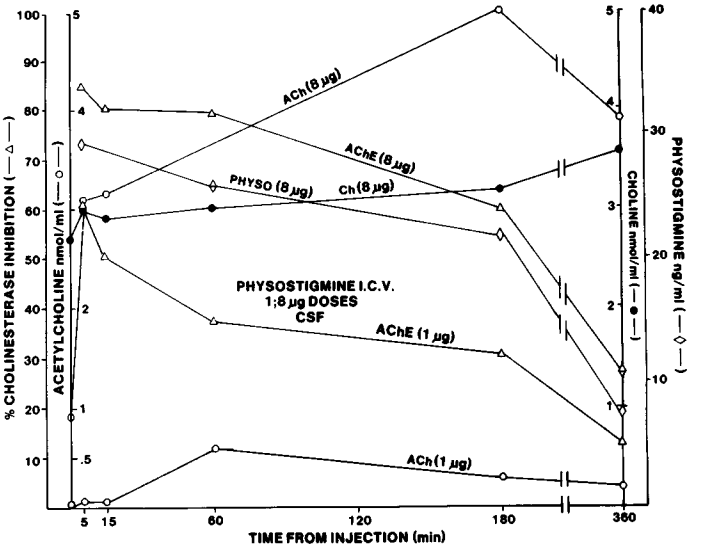



Figure 2: The effect of single doses of 1 or 8 µg Phy i.c.v. on AChE activity, ACh, Ch and Phy concentrations in CSF of an AD patient (from Giacobini et al., 1988c).

3. Neural Cholinergic Markers in CSF

Table VI summarizes the changes in cholinergic markers that correlate with a diagnosis of AD. The table synthesizes results from several investigations. Neural markers are from biopsy or autopsy material, and extraneural markers are from CSF, lymphocytes, erythrocytes, plasma and serum. The CSF is the the most accessible CNS compartment that can be exploited in the evaluation of the living patients and experimental animals. However, the use of CSF markers raises several questions regarding the origin of these markers and their relation to neuronal metabolism.

TABLE V
Cholinergic Deficits in AD Patients

| | |
|---|---|
| 1. | Scopolamine produces deficits in cognitive function in normal controls that resemble abnormalities seen in AD. |
| 2. | AD patients and demented Parkinsonians exhibit slowing of EEG activity that may be related to progressive cholinergic denervation of cerebral cortex. |

a. Acetylcholine

A review of CSF and ACh metabolism has been published recently (Giacobini, 1986). Levels of ACh ranging from 3 to 479 pmole/ml have been measured in human CSF. Judging from these data one may conclude that trace amounts (low picomole range) of ACh are probably present in normal human CSF. Following an i.c.v.

TABLE VI
Putative Cholinergic Markers of Alzheimer*

| | NEURAL | | NON-NEURAL | | | |
|---|---|---|---|---|---|---|
| | Cortical Biopsy or Autopsy | CSF | Lympho-cytes | Erythro-cytes | Plasma | Serum |
| Nicotinic Receptor binding | Decreased | | Decreased | | | |
| Muscarinic (post syn.) Receptor binding | No Difference | | Decreased | | | |
| ChAt Activity | Decreased | Not Measurable | | | | |
| AChE Activity | Decreased | Decreased | Decreased | Decreased but not. specific | AChE Increase BuChE No Difference or Increase | |
| Release of ACh | Decreased | Measurable after ChEI | | | | |
| Ach Synthesis | Decreased | | | | | |
| High Affinity Ch Uptake | Decreased | Increased Ch Levels | | | | |
| Altered Ch Metabolism | Decreased | Increased Ch Levels | | Normal or Increased Ch Levels | No Difference | |
| Cortisol | | | | | Increased | |
| Cortisol after Cholinergic drugs | | | | | Increased | |
| Antibodies to Cholinergic neurons | | Present | | | | Present |
| Anti-NGF Antibodies | | Present | | | | Increased |

*References for these studies are found in the text.

(intracerebroventricular) injection of Phy (8 μg) producing 85% inhibition of cholinesterase (ChE) activity in CSF, we found an increase in CSF ACh from 0.9 nmol/ml to a peak value of 5 nmol/ml at 180 min (Fig. 2). At 360 min, CSF ACh levels were still elevated (4 nmol/ml) despite only 30% CSF ChE inhibition (Giacobini et al., 1988c). Choline levels were stable, indicating that this precursor was not utilized to synthesize ACh in CSF.

We suggested that ACh may be released from the CNS into the CSF where its concentration is maintained at a high steady state level because of the sustained ChE inhibition. Do ACh and Ch found in human CSF reflect closely extracellular fluid concentrations in the brain as suggested by animal experiments (Tucek, 1984)? If so, high and sustained cerebral ACh in CSF could be used as a marker of the effect of Phy. Such marker would be important in order to establish the central effect of ChE inhibitors during therapy for AD.

b. Choline Levels and Acetylcholinesterase Activity

Choline is a precursor in the synthesis of ACh and of neuronal membrane phospholipids. Although the major source of brain Ch is exogenous, some Ch synthesis is present in brain and may play a role in AD (Blusztajn and Wurtman, 1983). We measured multiple Ch levels and acetylcholinesterase (AChE) activities in the CSF of 66 AD patients and 22 age-matched controls over a span of 24 months in order to study changes related to disease progression (Elble et al., 1989). We found a statistically significant reduction in AChE activity and an increase in Ch with advancing dementia. These changes were not related to age. We suggested that the rise in CSF Ch may be related to neuronal membrane breakdown and reduced uptake by cholinergic neurons. The reduction in CSF AChE activity is consistent with the depletion of cholinergic neurons in AD. In recent studies, we found that Ch levels but not AChE activity were significantly lower in patients with Parkinson disease, cerebellar ataxia, and Huntington disease, compared with healthy controls (Table VII). In AD, levels of Ch were also significantly lower in familial patients than in non-familial patients, but, there was no difference in AChE activity in these two groups (Kumar and Giacobini, 1988). It is noteworthy that Down's syndrome is the only other disease known to be associated with increased CSF Ch (Table VII) (Schapiro et al., 1989).

TABLE VII
Percent Changes in Levels of Choline and Acetylcholinesterase Activity in the CSF of Neurological Patients as Compared to Normal Controls

| Disease | Choline | AChE Activity |
|---|---|---|
| Alzheimer * | +60 | -30 |
| Down's Syndrome ***** | +28 | no difference |
| Parkinson ** | -56 | no difference |
| Huntington *** | -62 | no difference |
| Cerebellar Ataxia **** | -66 | no difference |

* Elble et al., 1988, 1989; ** Manyam et al., 1990a; *** Manyam et al., 1990b; **** Manyam and Giacobini, 1990; ***** Schapiro et al., 1990

Therefore, increased Ch and decreased AChE activity in CSF in AD may be important biochemical markers of neuronal damage and cholinergic dysfunction. Of particular interest is the relation of the biochemical finding to the progression and severity of the disease seen in AD (Elble et al., 1989).

c. Choline acetyltransferase

Early studies (Johnson and Domino, 1971; Haber and Grossman, 1980) reported a significant activity of choline acetyltransferase (ChAt) in the CSF, but, Aquilonius and Eckernas (1976) reported later that most activity was non-enzymatic. DeKosky et al. (1989) measured ChAt activity of different CSF fractions using the specific inhibitor NVP. They found that normal human CSF does not contain ChAt and that all ACh synthesis in CSF reflects non-enzymatically catalyzed synthesis.

4. Changes in Nicotinic Receptors in Alzheimer Disease

In Alzheimer and in other degenerative disease (Gerstmann-Straussler, Parkinson dementia of GUAM type and Dementia pugilistica), pathological and biochemical lesions in the cholinergic neuron are localized mainly to the presynaptic region. Alterations include biochemical and morphological changes in the structures storing, synthesizing and releasing ACh and possibly also in cholinergic receptors regulating ACh release. By comparing cholinergic terminals in the CNS of normally aged with AD patients, it is apparent that muscarinic and nicotinic receptors are not equally affected (Giacobini et al., 1988a, 1989; Zubenko et al., 1989; Rinne et al., 1989).

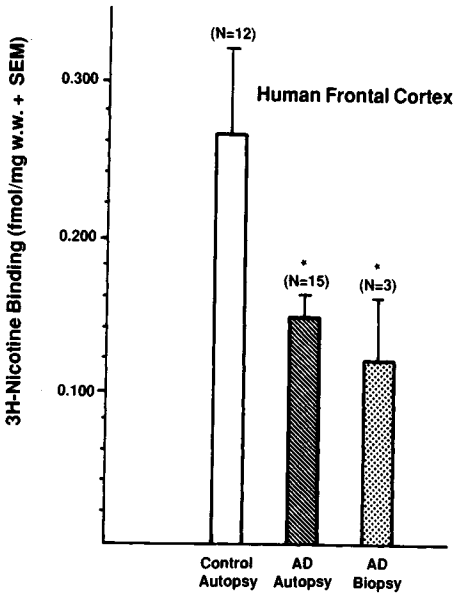



Figure 3: Comparison of $^{3}$H-nicotine binding (fmol/mg w.w. ± S.E.M.) in samples of human and frontal cortex from normal controls and Alzheimer patients (biopsies and autopsies). Significantly different from controls, $p < 0.05$.

Surveys, including percent changes in nicotinic receptors in the frontal cortex of AD patients, reveal a striking difference from aging normal controls (Giacobini et al., 1988a, 1989). Nicotinic receptor binding studies are mainly from autopsies using three different ligands [nicotine (NIC), α-bungarotoxin (α-BTX) and ACh]. Out of eight autopsy studies (1981-1988), including one from our laboratory (DeSarno et al., 1988; Fig. 3), six show decreases from 44-65% and two show no difference. Using $^{3}$H-quinuclinidyl benzylate ($^{3}$H-QNB) as ligand, we found no differences in specific binding in autopsy material but a significant 39% increase in the biopsies (Fig. 4). Brain muscarinic receptors are differently affected by AD and Parkinson disease (PD). A decrease in $M_2$-receptor binding is seen in the hippocampus of AD patients while PD patients have unaltered binding (Rinne et al., 1989). Using $^{3}$H-NIC as a ligand, we found a

44% decrease in the autopsies and a 55% decrease in the biopsies (Fig. 3). Preservation of the tissue might explain the differences between biopsy and autopsy material. However, in younger subjects it could reflect early changes in AD. These decreases in binding correlate to enzymatic (ChAt and AChE activity) changes (Davies, 1979; Rossor et al., 1982; Bird et al., 1983; Giacobini et al., 1988a,b, 1989) supporting a presynaptic localization of the lesion.

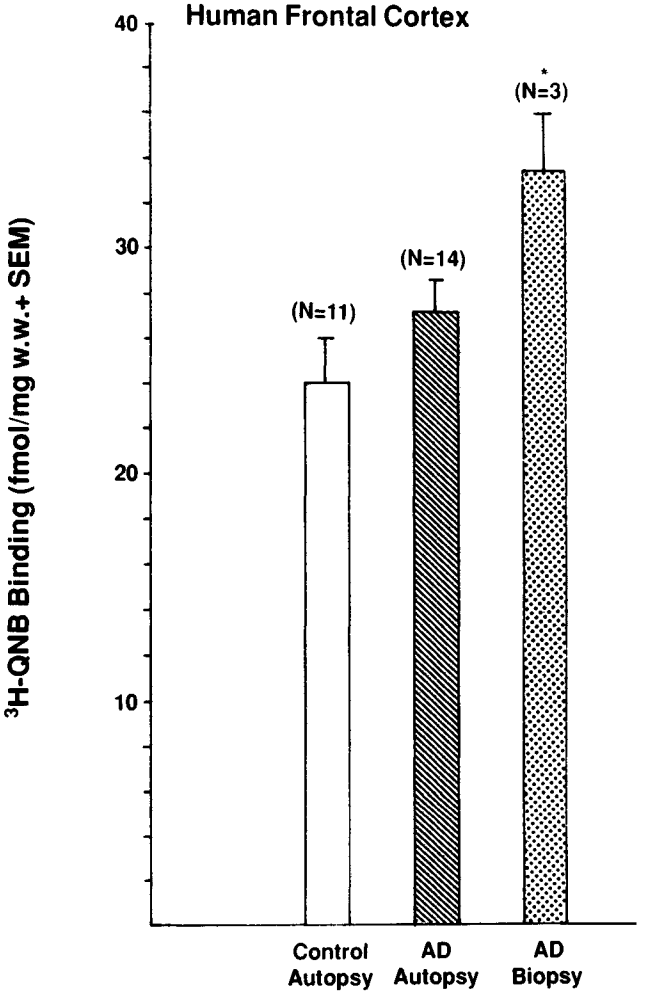



Figure 4: Comparison of $^3$H-QNB binding (fmol/mg w.w. ± S.E.M) in samples of human frontal cortex from normal controls and Alzheimer patients (biopsies and autopsies). *Significantly different from controls, $p < 0.05$.

The agonists used in these studies are not selective for either pre- or postsynaptic cholinergic sites. Therefore, it is difficult to say whether presynaptic sites on cholinergic axons are preferentially affected or postsynaptic sites on cholinoceptive neurons are also involved.

A second effect of decreased cholinergic receptor function is regulation of ACh release and the effect of drugs on this release. Release of $^3$H-ACh can be measured in frontal cortex slices from human autopsy after short post-mortem delay (Nordberg et al., 1987) and from biopsies (DeSarno et al., 1988; Giacobini et al., 1988a). A significant reduction (50-70%) in the evoked release of ACh has been reported by both laboratories. A reduction in ACh release and in number of nicotinic binding sites in the frontal cortex of AD patients supports the

hypothesis of a selective loss of presynaptic nicotinic receptors in AD (Giacobini et al., 1988a).

Although α-BTX has proved to be a useful cholinergic ligand in the peripheral nervous system, mainly at the neuromuscular junction, it is not suitable as a marker for the CNS (Morley et al., 1983). Experiments performed with iontophoretic techniques do not support the hypothesis that α-neurotoxins such as α-BTX bind to functional NIC receptors in mammalian CNS (Morley and Kemp, 1981; Chiappinelli, 1985). Consequently, characterization and quantitation of nicotinic cholinergic binding sites in the brain has met with difficulty. Recent studies have indicated differences between central and peripheral nicotinic receptors (Marks and Collins, 1982; Shimohama et al., 1985; Sugiyama and Yamashita, 1986). Chiappinelli (1985) and Wolf et al. (1987) have used a BTX that they named kappa-bungarotoxin (K-BTX) to characterize central nicotinic receptors in the chicken and in the rat. Kappa-bungarotoxin has been recently isolated from snake venom (Chiappinelli, 1985). It was shown to be a potent and selective antagonist of neuronal nicotinic receptors (Chiappinelli, 1985) including CNS sites in the chicken optic lobe (Wolf et al., 1987). We have observed that using $^{3}$H-NIC, $^{125}$I-α-BTX and $^{125}$I-K-BTX, at least three putative categories of nicotinic receptor are present in the human brain. Our studies suggest that each type of these receptors may have different kinetics, distribution and localization. Kappa-bungarotoxin or neuronal BTX has been shown to block nicotinic synaptic transmission in a variety of neuronal preparations where α-BTX has no effect. Vidal and Changeux (1989) have demonstrated that the effect of NIC applied by iontophoresis to the prefrontal cortex of the rat is blocked by K-BTX but not by other nicotinic antagonists. The agonistic actions of ACh in cerebellar neurons is also selectively blocked by K-BTX (de la Garza et al., 1989).

5. Nicotinic Receptor Subtypes in Human Cortex. Changes with Alzheimer Disease

The existence of several subtypes of nicotinic cholinergic receptors in the brain would suggest specific anatomical localizations and functions. In situ hybridization techniques suggest the presence of three independent nicotinic receptor subtypes in the rat brain (Heinemann et al., 1988).

Combining $^{3}$H-NIC, $^{125}$I-α-BTX and $^{125}$I-K-BTX as ligands, several categories of nicotinic receptors can be postulated in the human brain (Giacobini et al., 1988b). We reported the kinetics, distribution and localization of three subtypes present in the human frontal cortex. We also described for the first time specific changes related to receptor subtypes in human cortex of AD patients (Giacobini et al., 1988b).

We used autopsy brains from healthy young controls (age 21-57), and healthy elderly controls (age 64-94) as well as from AD patients (age 67-78) from our Regional Alzheimer Disease Assistance Center, for homogenate- or slice-in vitro assays of nicotinic binding. The right hemisphere was isolated and stored at -90°C for binding assays, the contralateral was fixed for histologically diagnosis.

Specific binding with increasing concentrations of $^{3}$H-(-)-NIC, $^{125}$I-α- or $^{125}$I-K-BTX to membranes of human frontal cortex was saturable. Scatchard plots were curvilinear and Hill coefficients far from unity, indicating the presence of multiple classes of binding sites for these three ligands.

There were significant decreases in the number ($B_{max}$) of high (47%) and low (50%) affinity binding sites of $^{3}$H-(-)-NIC in AD patients as compared to elderly controls (Table VIII). Alzheimer patients showed a significant decrease of $B_{max}$ in the low affinity binding site of $^{125}$I-K-BTX but not in the high

affinity site, as compared to young (57%) and elderly (52%) controls. There was only a non-significant decrease in $B_{max}$ in the low affinity binding site of $^{125}$I-α-BTX in AD patients, as compared to young and elderly controls (34.0% and 31.6%) (Table VIII).

TABLE VIII

Percent Decrease in Number of $^{3}$H-(-)-Nicotine, $^{125}$I-K- or $^{125}$I-alpha-Bungarotoxin Binding Sites in Frontal Cortex of Alzheimer Patients As Compared to Elderly Controls

| | High Affinity (% $B_{max}$) | Low Affinity (% $B_{max}$) |
|---|---|---|
| $^{3}$H-(-)-NIC | 47 | 50[a] |
| $^{125}$I-K-BTX | 0 | 52[a] |
| $^{125}$I-alpha-BTX | 0 | 32[b] |

n= 6-8; [a] significant $p < 0.005$; [b] not significant

Autoradiographic analysis showed that $^{125}$I-K-BTX specific binding sites are concentrated mainly in the middle and deep cortical layers. The same localization has been observed by us in the monkey (Macacus rhesus). With autoradiography, a pronounced decrease in density of $^{125}$I-K-BTX binding sites was seen in AD patient vs. elderly controls.

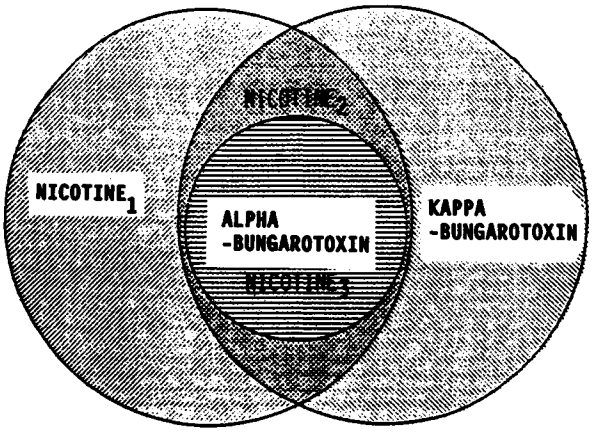
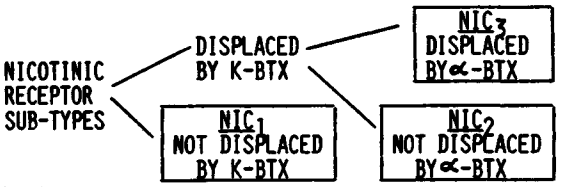



Figure 5: Diagram of subtypes of nicotinic receptors in human frontal cortex demonstrated by using nicotine, α-bungarotoxin and K-bungarotoxin as ligands. See text.




The kinetic characteristics of these three binding sites were studied by means of competition experiments. These demonstrated the presence of two categories of nicotinic binding sites, one which was displaced by K-BTX and one which was not displaced by this toxin (Fig. 5). We named the latter Nicotine$_1$

(Fig. 5). The binding site not displaced by K-BTX could be subdivided into two subtypes, one which was displaced by α-BTX and one was not. We named these two binding sites $Nicotine_2$ and $Nicotine_3$, respectively (Fig. 5).

A functional role of cortical nicotinic receptors can be demonstrated by the effect of BTXs on the evoked fractional release of ACh from rat frontal cortex slices. In these experiments, the first electrical stimulation ($S_1$, 20 mA, 1 Hz, 5 min) is a pre-drug testing control and the third electrical stimulation ($S_3$) is a post-drug testing control. The only significant decrease was seen in the $S_2/S_1$ ratio of ACh release following 1 μM K-BTX but not after 1 μM α-BTX. The $S_3/S_1$ ratio did not change after the third stimulation. This shows that K-BTX, but not α-BTX, decreases the electrically evoked release of ACh from rat frontal cortex slices and that this effect is reversible.

Mapping of nicotinic receptors in rodent brain, using various radiolabelled agonists (Clarke et al., 1985), in situ hybridization (Goldman et al., 1987) and immunohistochemistry (Swanson et al., 1987) has demonstrated the presence of a high number of ACh receptors in the neocortex. Our data on human brain autoradiography of K-BTX binding sites also show the highest density in the cortex. We have seen variable regional distributions in the presence of (-)-NIC, α-BTX or K-BTX. Receptor subtypes in human cortex have been previously suggested based on various ligands such as $^3$H-ACh, $^3$H-(-)-NIC and $^{125}$I-α-BTX (Adem, 1987). The regional distribution and the pharmacology of agonists and antagonist binding sites suggest that these subtypes may be present in different neuronal populations in pre- and post-synaptic locations.

Our results show that two major categories of nicotinic receptors, one K-BTX insensitive and the other one K-BTX sensitive are present in human cortex (Fig. 3). Our experiments show that α-BTX displaces high (100%) and low (25%) affinity binding sites of K-BTX but does not decrease the electrically evoked release of ACh. The K-BTX sensitive subtype has an α-BTX binding site which includes the high affinity binding site of K-BTX. The other subtype does not have an α-BTX binding site and modulates ACh release from rat frontal cortex through a K-BTX sensitive nicotinic receptor. Thus, our data are in agreement with the molecular biological data on receptor gene families in mammalian species (Heinemann et al., 1988; Lindstrom et al., 1988) suggesting that human cortex has at least three different subtypes of nicotinic receptors, each showing specific kinetics, regional distribution and synaptic localization. These three subtypes are represented in the human frontal cortex. In addition, we have found that parietal, temporal lobe and hippocampus exhibit the same binding sites with a different receptor density and distribution. Hippocampal cortex (CA3) show the highest density in K-BTX binding sites.

The fact that K-BTX, but not α-BTX, decreases the electrically evoked fractional release of ACh in rat brain is a strong indication for a presynaptic localization of K-BTX binding site. This agrees with the finding of Vidal and Changeux (1989) who demonstrated an antagonism of K-BTX (1.4 μM) to stimulation by iontophoretically applied NIC in slices of rat prefrontal cortex. In these experiments none of the classical nicotinic antagonists and α-BTX was found to have any effect on nicotinic responses.

EXTRANEURAL CHOLINERGIC MARKERS

a. Choline in Red Blood Cells and Plasma

Sizeable evidence suggests that cells outside the CNS may express pathological changes related directly or indirectly to AD (Blass and Zemcov, 1984). Some of these changes are related to alterations in cell membrane composition and function such as an increase in membrane fluidity in platelets (Zubenko et al., 1987) and altered concentrations and transport of Ch phospho-

lipids in red blood cells (RBC), lymphocytes and plasma. RBC Ch was found to be elevated in four published studies (Blass et al., 1985; Domino et al., 1986; Greenwald et al., 1985; Miller et al., 1986; Sherman and Friedman, 1986). Sherman et al. (1986) found that plasma Ch was significantly but equally elevated in elderly controls and AD patients compared to young controls. Choline uptake in RBC was also increased in both elderly controls and AD patients (Sherman and Friedman, 1986). Thus, these age-dependent abnormalities were not exacerbated by AD (Table VI).

Greenwald et al. (1985) found that mean values for RBC Ch, plasma Ch and the ratio RBC/plasma Ch did not differ between AD patients and controls. By contrast, Miller et al. (1986) found that mean RBC Ch was elevated in AD. These contrasting results do not support the use of RBC and plasma Ch as a diagnostic tool or as an index of cholinergic deficits in AD (Table VI).

b. Blood Acetyl- and Butyrylcholinesterase

A number of investigations have dealt with ChE activities in blood of AD patients. In human blood, plasma ChE is mainly of the butyryl type (BuChE), but the erythrocyte enzyme is mainly AChE (Elble et al., 1987). Chipperfield et al. (1981) found that AChE activity was decreased in RBCs of AD patients. Perry et al. (1982) also found that RBC AChE was decreased in AD patients while plasma BuChE activity was increased and similar reductions in RBC AChE activity were found in depressed patients (Perry et al., 1982). Smith et al. (1982) found that BuChE activity in plasma was about 100% higher in AD patients than in age-matched controls while RBC AChE activity tended to be lower. Atack et al. (1985) measured both AChE and BuChE activity in plasma but found that only AChE activity was elevated in AD. They found no difference in RBC AChE or plasma BuChE activity. It was suggested that part of AChE activity in plasma may originate from the CNS and that elevated AChE activity levels reflect increased release or leakage of the enzyme from degenerating cholinergic neurons. The decrease in AChE activity observed in CSF of AD patients could result from leakage of the enzyme from CNS to plasma. St. Clair et al. (1986) used a monoclonal antibody raised against RBC AChE to measure AChE molecular forms in the presence of BuChE activity without using inhibitors. No difference was found in RBC or plasma AChE activity in AD, multi-infarct dementia, Huntington and Korsakoff patients and normal controls. Adem et al. (1986), using butyrylthiocholine as a substrate did not observe any change in BuChE activity in AD patients, whereas a significant decrease was found in PD patients. Lymphocyte AChE activity has been measured in AD by Bartha et al. (1987) and found to be significantly lower in AD. These studies, taken together, do not suggest that peripheral AChE or BuChE activities measured in RBC or plasma, reflect altered cholinergic function in AD (Table VI).

c. Cholinergic Receptors on Lymphocytes

Adem et al. (1986) observed a significant decrease in both muscarinic and nicotinic binding sites in lymphocytes from AD patients while in PD patients a significant decrease was seen only in nicotinic binding sites. Rabey et al. (1986) found that in lymphocytes derived from normal human subjects, muscarinic binding capacity increased with age. In contrast, lymphocytes from AD patients exhibited a marked reduction in binding capacity. Treatment with antimuscarinic drugs was associated with increased muscarinic binding by lymphocytes. Ravizza et al. (1988) found that muscarinic receptor binding to lymphocytes with $^{3}$H-NMS (N-methyl scopolamine) was decreased in AD but not in PD patients. In conclusion, several studies suggest that cholinergic receptor binding to lymphocytes might aid in the diagnostic profile of AD. Furthermore, it might provide a valuable tool to monitor the progression of the disease (Table VI).

d. Plasma Cortisol and Cortisol Responses to Cholinergic Drugs

Davis et al. (1986) found that AD patients with a senile onset of dementia had a significantly higher concentration of cortisol in plasma than those with presenile onset. CSF nocturnal cortisol and MHPG (3-methoxy-4-hydroxyphenyl-glycol) concentrations were negatively correlated. Cortisol concentrations at 9 AM were significantly higher in AD patients than in normal subjects. The 9 AM cortisol concentration correlated with severity of dementia but not with age. In order to investigate changes in plasma cortisol levels produced by central cholinergic stimulation due to increased levels of ACh in brain, Kumar et al. (1988) studied the differential effect of two ChE inhibitors, Phy and neostig-mine, in a double-blind crossover investigation of AD. Physostigmine (60 $\mu g/kg$) increased plasma, cortisol relative to neostigmine (7.5 mg) (a peripherally acting inhibitor that does not penetrate the CNS) with the greatest difference more than 90 min after oral administration. Physostigmine also significantly decreased plasma BuChE activity in the same patients. There was a significant positive correlation between the increase in cortisol and the decrease in ChE activity due to physostigmine but not to neostigmine (Table VI).

An increase in plasma cortisol produced by oral or i.v. Phy administration has also been reported by Risch et al. (1980), Davis et al. (1982) and Mohs et al. (1985). These results suggest that secretion of cortisol after Phy is due to increased central cholinergic activity and that measurement of cortisol in plasma following challenge with ChE inhibitors could be used as an indicator of central cholinergic activity during treatment of AD with cholinomimetic drugs.

e. Antibodies in CSF and Serum that Recognize Cholinergic Neurons and NGF

Discovery of immunological factors circulating in the blood or present in the CSF of AD patients would suggest immunological reactions related to the pathogenesis of the disease. Antineuronal antibodies have been measured in the serum of AD patients by Ingram et al. (1974) and Mayer and Cape (1976). In the CSF of a subgroup of AD patients, antibodies were found that recognize cholinergic neurons in the rat CNS (McRae-Degueurce et al., 1987). These antibodies specifically bind to neuronal populations in the medial septum and spinal motor neurons. The CSF immunocytochemical reaction is blocked by a rabbit anti-ACh antiserum. Other authors have also found antibodies to cholinergic neurons to be present in serum of AD patients (Chapman et al., 1988). These results suggest that decreases in cholinergic neurons or presynaptic cholinergic markers in AD could be triggered by abnormalities related to the immune system. An antibody that bound to submaxillary gland 2.5S nerve growth factor (NGF) and 7S NGF was recovered in the serum of 3/5 of patients with AD. Such binding was inhibited by soluble 2.5S NGF (Roy et al., 1988). In our laboratory, Downen et al. (1988) found antibodies in human serum and CSF that recognize NGF from mouse submaxillary gland. When serum anti-NGF was plotted as a function of age in normal controls and AD patients (55-92 yrs), there was a tendency for anti-NGF antibodies to increase with age in AD but not in normal controls. There was no difference in the amount of anti-NGF Ig in serum and CSF of AD patients as compared to normal controls of corresponding age, but in those patients with greater serum anti-NGF activity, there was greater anti-NGF in the CSF.

Anti-NGF antibodies found in human serum and CSF that bind to submaxillary gland 7S NGF need to be further characterized. These findings are of special interest since cholinergic neurons having NGF-receptors on their cell bodies and dendrites are selectively affected by AD (Hefti, 1983; Hefti and Weiner, 1986). However, investigations measuring changes of NGF levels or NGF mRNA levels in postmortem tissue of AD patients has proven inconclusive.

GENERAL CONCLUSIONS

As summarized in Table VI, several neural and non-neural markers of cholinergic function correlate with a diagnosis of AD. However, none of these markers is singularly diagnostic. In order of importance, we would rank first data from biopsy indicating a decrease in number of cholinergic receptors in cortex, particularly presynaptic nicotinic receptors, enzyme activities related to ACh synthesis (ChAt), and hydrolysis (AChE) and release of ACh. In CSF, decreased AChE activity and increased levels of Ch are the only two indexes that correlate with the severity of dementia. Among several extraneuronal markers the presence of antibodies to cholinergic neurons, if confirmed, is of particular interest. In the future, new markers may be developed which might be more selective for presynaptic changes. These include new and more specific ligands for nicotinic receptors and the possibility of in vivo visualization of these receptors with PET-scanning.

ACKNOWLEDGEMENTS

The authors wish to thank Elizabeth Williams and Constance Higgins for their technical assistance and Diana Smith for typing and editing the manuscript. Supported in part by National Institutes of Aging AG05416.

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# Part 4

# Electroencephalogy



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# EEG in the Diagnosis of Early Alzheimer Disease

H. Soininen, P. Riekkinen, V.J. Partanen, A. Pääkkönen,
E-L. Helkala and V. Laulumaa
*Departments of Neurology and Clinical Neurophysiology*
*University of Kuopio, Finland*

## INTRODUCTION

According to the clinical criteria of 'probable' Alzheimer's disease (AD), the diagnosis is supported by a normal pattern or nonspecific changes in the EEG such as slow wave activity (McKhann et al., 1984). EEG has been a traditional method in the diagnosis of dementia (Nuwer, 1988b). Several studies using both conventional and quantitative EEG analysis have shown the slowing of the EEG in AD (Johanneson et al., 1979; Stigsby et al., 1981, Soininen et al., 1982a; Coben et al., 1983; Duffy et al., 1984a; Penttilä et al., 1985; Brenner et al., 1986). EEG alterations have been described to parallel the cognitive decline in AD (Kazniak et al., 1979; Penttilä et al., 1985). Most of the studies showing a relationship between EEG changes and severity of neuropsychological deficits have been based on different patients at different stages of dementia. A few follow-up studies have further evaluated EEG dynamics during the progression of the disease (Coben et al., 1985, Rae-Grant et al., 1987).

When the value of EEG examination in the early stages of AD is considered, the major questions to be answered are: 1. How reliable is EEG in the diagnosis of mild disease? 2. Has EEG any prognostic value? 3. Can EEG reflect the neurochemical pathology such as the cholinergic deficit of the brain and help to select patients for drug trials? Our approach to EEG dynamics in AD started with studies using conventional EEG in AD patients with advanced disease (Soininen et al., 1982a). Later, quantitative EEG methods have been applied to evaluate EEG changes at different stages of AD (Penttilä et al., 1985). Recently, a follow-up study of AD patients has increased our knowledge about EEG changes at the early stages and about alterations as function of deterioration of the disease (Soininen et al., 1989a).

### EEG changes in normal aging

Slight EEG alterations are common in normal elderly people after the age of 60 years. The main changes are slowing of the alpha rhythm, appearance of slow activity and a theta focus in left

temporal region. However, the changes in EEG associated with aging are quite modest. Duffy et al., (1984b) suggested that the previously reported age-related alterations may be due to the presence of disease in the population studied. On the other hand, both animal and human studies indicate that aging is associated with neurotransmitter changes such as reduced cholinergic activity of the cortex (Reinikainen et al., 1988, Sirviö et al., 1988). In animals, the slowing of the EEG has been induced with anticholinergic drugs and with lesions of the nucleus basalis of Meynert (Vanderwolf, 1975; Stewart et al., 1984; Buzsaki et al., 1988).

Although the role of the cholinergic system in the regulation of thalamocortical and neocortical information processing and EEG is crucial, there is increasing evidence that depletion of the noradrenergic and serotoninergic systems in lesions, normal aging or in pharmacological modulation will induce neocortical EEG changes (Vanderwolf, 1987; Vanderwolf and Stewart, 1988). Furthermore, there is evidence that these ascending pathways reveal shrinkage in neurons of the nucleus raphe and locus coerulaeus, respectively, in certain proportions among old animals and also in human autopsy material (Arnsten and Goldman-Rakic, 1985; Reinikainen, 1988).

In a study of 84 elderly normal individuals, conventional EEG showed changes in 29% of the subjects (Soininen et al., 1982a). Abnormalities were mild, the most common alteration being asymmetic findings, mainly theta activity in the left temporal region . In another study using quantitative EEG analysis, 4 out of 14 normal ederly controls showed 1 abnormality (score 0-4) (Soininen et al. 1989b). In that study, the EEG was concidered normal if the relative alpha power is more than 50%, the theta and delta percentage less than 15% and the mean frequency values higher than 8 Hz.

## EEG alterations in Alzheimer's disease

EEG changes associated with AD consist of more slowing of the dominant occipital rhythm and more accentuation of theta and delta activity than is found in normal old people, but local slowing occurs rarely (Soininen et al., 1982a). It is evident that the reliability of EEG in differentation between dementia and normal aging and the best discriminating parameter vary according the stage of dementia.

In a study of 62 AD patients (24 severe, 18 moderate and 20 mild cases) and of 84 normal elderly people, in discriminant function analysis of EEG variables: dominant occipital rhythm, accentuation of theta and delta, fluctuations in alertness, asymmetric findings, paroxysmal activity, H-reaction in photostimulation, age and sex, the highest coefficient was for the dominant occipital rhythm (Table 1). Using this analysis 82% of the patients and 89% of the controls were correctly classified (Soininen et al., 1982b).

Table 1. Discriminant function coefficients and their standard errors for EEG variables between patients with Alzheimer's disease age-matched controls.

| | Coefficient | Standard error |
|---|---|---|
| Dominant occipital rhythm | -0.634* | 0.026 |
| Accentuation of theta and delta | +0.409* | 0.040 |
| H-reaction | +0.385* | 0.038 |
| Paroxysmal activity | -0.119 | 0.060 |
| Fluctuations in alertness | -0.022 | 0.042 |
| Asymmetric findings | +0.006 | 0.036 |
| Sex | +0.065 | 0.063 |
| Age | -0.138 | 0.004 |

* $p<0.05$

Brenner et al. (1986) compared quantitative EEG findings of AD patients, patients with major depression and normal elderly people. Compared to controls, demented patients had a significant increase in the theta and alpha bandwidths, as well as an increased theta-beta difference. The parasagittal mean frequency and beta activity were significantly decreased. In demented patients, there was a high correlation between several EEG parameters (parasagittal mean frequency, delta and theta activity, and the theta-beta difference) and the Mini-Mental score (MMS). Using the mean frequency to differentiate dementia patients from controls, specificity of 93% and sensitivity of 66% could be achieved. Sensitivity was higher, 79%, for patients with MMS 22 or less, whereas for patients with milder disease (MMS over 22) sensitivity was only 36%.

A study by Coben et al. (1983) points out that in mild AD the significant differences are accentuation of theta as well as decrease of beta activity and of the average mean frequency compared to matched normals. In a longitudinal study of 2.5 years, Coben et al. (1985) found that the relative theta power distinguished between all 4 stages, control, mild, moderate and severe dementia. In the moderate stage the alterations seen in mild disease persisted, and the alpha became different. Delta was not increased until the severe stage of AD.

Our results of a study (Penttilä et al., 1985) on changes in quantitative EEG in 42 patients with presumptive diagnosis of AD at different stages of cognitive deterioration agree with findings of Coben and coworkers (1983). When the percentage powers of the alpha, theta and delta bands were compared, it was discovered that the theta power increased in mild AD. The most marked increase in the delta power did not occur until severe AD. The decrease in the alpha power was significant in moderate but not mild AD. The alpha/theta ratio decreased abruptly in mild AD, whereas the

alpha/delta ratio declined more linearly, and the difference was significant in marked and severe AD compared to control values. The occipital peak frequency was in the alpha band in mild and moderate AD, and it was not significantly decreased in mild disease, whereas the mean frequency was significantly decreased already in mild AD. There was a significant correlation between the peak frequency and the neuropsychological test score ($r=0.60$, $p<0.001$). Thus according to our observations, the best variables diffentiating mild AD from controls are the percentage power of the theta band, alpha/theta ratio and the mean frequency in the range 1.5-20.0 Hz. It is evident that the slowing of the dominant occipital rhythm and accentuation of diffuse irregular slow waves describe rather advanced but not mild AD.

**Longitudinal quantitative EEG**

In order to get better understanding of the pathophysiology of AD, we have conducted a follow-up study of AD patients. Thirty patients meeting the criteria of 'probable' AD according to the definition of the NINCDS-ADRDA work group (McKhann et al., 1984) were recruited to the study at the early stage of dementia. After the diagnostic work-up including clinical neurological exmination, neuropsychological tests evaluating different aspects of cognition, laboratory blood and CSF studies, conventional and quantitative EEG and CT scan of the brain, the patients are carefully followed as long as they live. Ultimately, the diagnosis will be confirmed at autopsy in as many cases as possible. The clinical and neuropsychological examinations are performed every half a year, and EEG and CSF neurotransmitter-related parameters are studied once a year. One of the aims of the study is to evaluate the relationship between brain electric activity and neuropsychological deficits in AD patients as well as possible neurochemical substrates of EEG changes.

Preliminary results of baseline and 1 year EEG data of 24 AD patients has been published and the EEG method described in detail elsewhere (Soininen et al., 1989a,b). A conventional EEG was recorded using a 10- or 16-channel Siemens Mingograph apparatus and international 10/20 system. The EEG from the derivation T6-O2 (or T5-O1) was recorded in parallel but separately. The records were amplified and filtered with bandpass set at 0.5-50.0 Hz. The EEG was digitized at 125 samples/s. Four epochs of 8.1 s were recorded, transferred to a computer and stored on a disc for off-line Fast Fourier Transform analysis. The absolute and relative (percentage of the total EEG power) powers in the delta (1.46-3.91 Hz), theta (4.15-7.32 Hz), alpha (7.57-13.92 Hz) and beta (14.16-20.02 Hz) bands as well as peak and mean frequences were calculated. The statistical analysis was done using Student's t-test for independent and paired samples. To change the data into Gaussian distribution, a logarithm transformation, $\log(x/(1-x))$, was applied for relative power values, where log is the natural logarithm and x the relative power of a frequency band (Gasser et al., 1982).

When the EEG findings of AD patients at baseline, at the time

Figure 1. * p<0.01

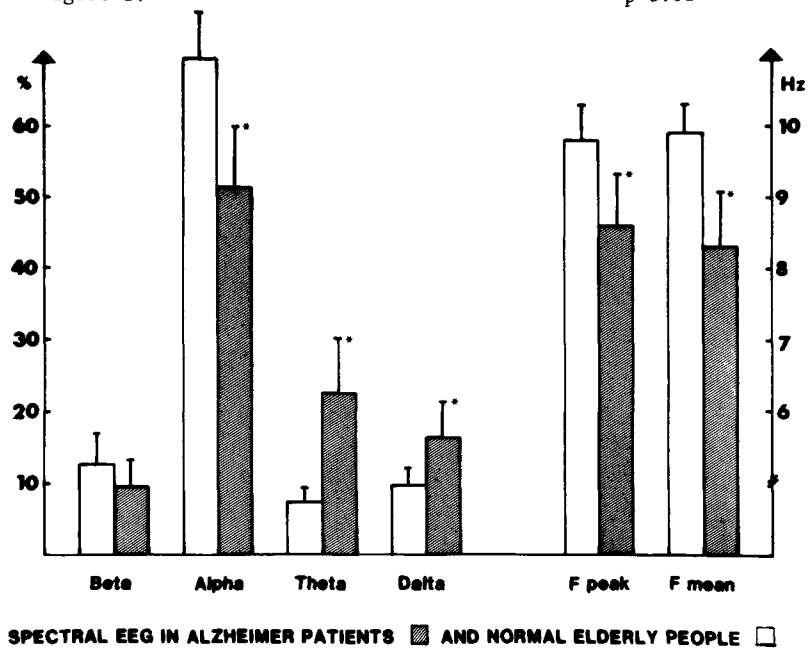
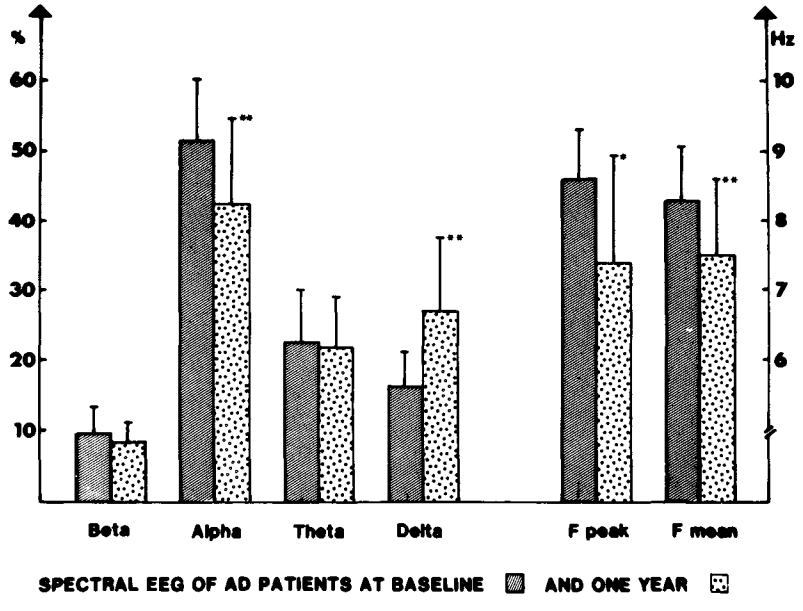



Figure 2. * p<0.05
** p<0.01



the diagnosis was made, were compared to control values, there was a significant difference in all relative power values except beta power. Furthermore, the peak and mean frequency were significantly lowered (Figure 1). As to absolute power values, there is a remarkable variability both for the controls and for the patients. However, the AD patients had significantly higher absolute theta power ($t=-2.75$, $p=0.01$) compared to controls. Moreover, absolute beta power tended to be lower for the AD patients than for the controls ($t=2.46$, $p=0.02$), whereas absolute delta and alpha powers were unaltered.

When the EEG variables of 24 AD patients at baseline and at 1 year were compared, EEG deterioration was reflected in the relative and absolute alpha power, in the relative delta power as well as in the mean frequency (Figure 2 shows relative powers and frequency parameters). The absolute and relative beta and theta power and the peak frequency were unchanged. However, when individual patients were considered, the deterioration of the EEG was evident in 12 (50%) of the patients. Four of them fulfilled all 4 criteria of deterioration, 5 fulfilled 3 criteria and 3 fulfilled 2 criteria. The EEG was considered to deteriorate if at least 2 of the following criteria were fulfilled: decrease of the alpha power more than 10%; increase of the theta power more than 10%; icrease of the delta power more than 10%; decrease of the mean frequency more than 1 Hz. In 9 of these 12 patients also the conventional EEG showed general disturbance and also the remaining 3 patients with initially normal EEGs, showed general disturbance at 1 year. In the EEGs of the other 12 patients, 11 did not fulfill any of the criteria of deterioration and one fulfilled one criterion (decrease of the alpha power more than 10%). In the conventional EEG, 10 of these patients had a normal finding and 2 had general disturbance. There was no obvious change in the EEGs of these patients at 1 year.

The patients with deteriorating and stable EEGs did not differ in age, age at onset of the disease, duration of the disease, clinical severity estimated by Clinical Dementia Rating (Hughes et al., 1982) and Brief Cognitive Rating Scale, BCRS, (Reisberg et al., 1983) or use of CNS active medication. The BCRS scores for the subgroup with stable and deteriorating EEGs were at baseline $28.4\pm6.1$ and $28.8\pm7.1$ and at 1 year $35.1\pm7.0$ and $35.1\pm9.0$, respectively. Both subgroups showed significant clinical progression of the disease (Wilcoxon; $p=0.0173$, $p=.0077$, respectively) during 1 year.

In the neuropsychological test battery including WAIS verbal and performance IQ, cortical functions (praxia of the hand, automatic speech, understanding of speech and visual functions) evaluated by Luria's neuropsychological examination, memory tests (a shopping list learning test with the Buschke selective reminding method) and alternating motor functions (drawing every second S-letter reversed), the only difference at baseline was observed in the alternating motor task. The patients with deteriorating EEG had more perseveration in this test. In the follow-up of 3 years,

however, the patients with worsening EEG have shown more disturbed parietal functions, such as naming and praxia of the hand, compared to the patients with normal EEG but memory functions were equally disturbed in both subgroups (Helkala, unpublished observation). Interestingly, also at baseline the delta activity correlated with several cortical functions e.g. praxia of the hand, visual functions, automatic speech and understanding of speech but not with memory functions (Helkala, unpublished observation). The extensive damage of the hippocampus responsible for the memory disorder in AD (Hyman et al., 1984) does not necessarily cause changes in the scalp EEG. On the other hand, the widespread defect of the neocortical cholinergic innervation which has been shown to cause EEG slowing in animals (Stewart et al. 1984), can be expected to result in several cognitive deficits. Further follow-up of the patients will give more information about the prognostic value of EEG slowing in terms of deterioration of cognition and in terms of life expectancy.

## Cholinergic deficit and EEG slowing

Though AD is associated with several neurochemical alterations, the most consistent change in brains of AD patients is the deficit of the cholinergic system (Bird et al., 1983; Davies, 1983; Rossor et al., 1986; Palmer et al., 1987). Reduction of the presynaptic cortical cholinergic markers and the loss of neurons in the nucleus basalis of Meynert have been confirmed in numerous studies (Whitehouse et al., 1981; Reinikainen et al., 1988). Both pharmacological and lesion studies suggest that the ascending cholinergic projections from the nucleus basalis to the neocortex play a major role in the regulation of brain electric activity (Vanderwolf, 1975; Steward et al., 1984; Buzsaki et al., 1988). Reduction of the cholinergic drive to the cortex with anticholinergic drugs, scopolamine or atropine, or lesions of nucleus basalis have induced EEG slowing. Thus it is interesting to find out whether there is any relationship between cholinergic markers and EEG slowing in AD. On the other hand, there is evidence supporting the contribution of the monoaminergic system to EEG activity (Vanderwolf, 1984). Interestingly, a correlation analysis between quantitative EEG variables and a cholinergic marker (AChE activity of the CSF) and CSF monoamine metabolite concentrations (homovanillic acid, HVA; 5-hydroxyindoleacetic acid, 5-HIAA; 3-methoxy-4-hydroxyphenylglycol, MHPG) of AD patients, showed a significant negative correlation between the delta power and the CSF AChE activity ($r=-0.57$, $p<0.001$, $n=23$) (Riekkinen et al. 1989). Monoamine metabolites did not correlate with any of the EEG parameters.

There is no reliable and easily accessible cholinergic marker for use in life-time in studies of AD patients (Appleyard et al., 1987; Arendt et al., 1984; Reinikainen et al., 1988). The AChE activity of the CSF, as a marker of the degeneration of the nucleus basalis, has several limitations. The origin of the AChE acitivity in lumbar CSF is not exactly known. The AChE activity is not only limited to cholinergic neurons but can be found also in

noncholinergic neurons and even in nonneuronal structures (Greenfield, 1984). However, the observed relationship between the delta power and the CSF AChE values in AD is in line with experimental pharmacological and lesion studies which have showed a relationship between the damage of the nucleus basalis and the EEG slowing. Interestingly, preliminary post-mortem findings have shown that the patients with the highest delta power had the lowest cell counts in the nucleus basalis and the lowest choline acetyltransferase activity of the cortex (Riekkinen, unpublished observation).

Despite the importance of the cholinergic system in the regulation of neocortical EEG activity, the contribution of noradrenergic and serotoninergic defects to EEG changes in AD cannot be excluded. Correlation of life-time EEG alterations to post-mortem neurochemical findings and pharmacological modulation of EEG in patients and in aged and lesioned experimental animals will expand our understanding of the generation of brain electrical activity and EEG changes associated with AD.

## SUMMARY

The reliability of EEG in the diagnosis of Alzheimer's disease (AD) depends on the stage of the dementia. In the early stages of the disease, even 50 % of AD patients may have a normal conventional and quantitative EEG with no worsening of the EEG a year later suggesting the heterogeneity of the disease. The best parameters of the quantitative EEG distinguishing mild AD from normal aging are the relative and absolute theta power, alpha/theta ratio and the mean frequency in the range 1.5-20.0 Hz. The relative alpha power, occipital peak frequency and alpha/delta ratio decrease linearly in different stages of AD. The distinct slowing of the occipital peak frequency and distinct accentuation of the delta power occur in advanced disease. A correlation between the increased delta power and the decreased CSF acetylcholinesterase activity suggest that the amount of delta may reflect the extent of the cholinergic deficit in AD. There is also preliminary autopsy evidence that the damage of the nucleus basalis of Meynert in AD is correlated to the EEG slowing. However, the role of the noradrenergic and serotoninergic system in the regulation of the neocortical EEG must be studied in pharmacological models, before their role can be excluded.

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# Part 5

# Neuropharmacology



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# Drug Challenge Strategies in Alzheimer Disease:
## A Focus on the Scopolamine Model

Trey Sunderland, Susan Molchan, Rick Martinez
and Benedetto Vitiello
*Unit on Geriatric Psychopharmacology, LCS, NIMH, Bethesda, MD*

Peter Martin
*Division of Alcohol and Substance Abuse, School of Medicine*
*Vanderbilt University, Nashville, TN*

**INTRODUCTION:**

Considering drugs or other exogenous substances as challenge agents is quite common in medical practice, but this strategy is relatively new to biologic psychiatry research in general, and Alzheimer's research in particular. The scopolamine model is one example of such a challenge strategy. Scopolamine testing is based on the well-founded assumption that an underlying cholinergic abnormality exists in Alzheimer patients compared to controls, and the contrasting fact that not all Alzheimer patients respond therapeutically to pro-cholinergic agents when they are administered in research trials. While there are other current and potential challenge strategies for Alzheimer's patients, we will focus on the scopolamine model because it is perhaps the most tested thus far, and because it's advantages and disadvantages are more readily apparent.

Before proceeding further, however, it is important to distinguish between a challenge paradigm and the more common therapeutic drug testing procedures. The scopolamine model, and others like it, are intended to test the underlying sensitivity of targeted receptor (i.e. cholinergic) and other effector systems (i.e. neuroendocrine responses or behavior). While the challenges may relate in some fashion to eventual therapeutic responses, they should not be confused with acute or chronic therapeutic drug trials. Simply put, a scopolamine challenge or, for that matter, a test dose with the cholinergic agonist arecoline, cannot be thought of as a mini-trial of that agent any more than a one day trial of amitryptyline can be thought of as a short course of antidepressants. The neuropharmacology of acute versus chronic exposure is quite different, and the goals of the tests are generally

distinct. The scopolamine paradigm is therefore designed as a research test and has little or nothing to do with the therapeutic efficacy of other agents.

**The Cholinergic Theory of Alzheimer's Disease:**

Interest in cholinergic treatment in Alzheimer's disease stems from two large but distinct bodies of data. First, the acetylcholine system has long been implicated in animal and human memory (Olds and Domino 1969; Deutsch 1971; Drachman and Leavitt 1974; Bartus et al. 1976; Flood and Cherkin 1988). Second, Alzheimer's disease, widely recognized clinically by the hallmark symptom of memory loss, has been associated with multiple deficits in the brain's cholinergic neurotransmitter system, among others (Davies and Maloney 1976; Whitehouse et al. 1982; Arendt et al. 1983; Francis et al. 1985; Collerton 1986). These converging data have led many investigators to test therapeutic replacement strategies with various cholinergic agents (Chistie et al. 1981; Brinkman et al. 1982; Davis and Mohs; Summers et al., 1986). These therapeutic trials have, for the most part, been only modestly successful, leading investigators to question the unique importance of the cholinergic hypothesis in Alzheimer's disease. While it is now recognized that there are multiple non-cholinergic deficits in the brains of Alzheimer patients, including abnormalities in the serotonergic, noradrenergic somatostatinergic, and other peptidergic systems (Francis et al. 1987; Bowen et al. 1983; Bondareff et al, 1982; Davies et al. 1980; Cross et al. 1986; Davies 1988), the single biggest deficit remains in the cholinergic system. For this reason, attempts to model the abnormalities found in Alzheimer's disease have centered mostly around cholinergic pharmacologic challenges.

**Modelling Memory Impairment with Scopolamine:**

Scopolamine is a potent, centrally-active antimuscarinic agent. When given in sufficient doses to animals, it can cause a temporary blockade of central muscarinic cholinergic receptors and is associated with a readily apparent memory impairment (Bartus and Johnson 1976; Flood and Cherkin 1986). Scopolamine has been administered to many species of animals employing various learning paradigms (see Table 1). In most cases, the experimental findings are consistent with a reversible, pharmacologically-induced deficit in new learning which is analagous, at least in part, to some of the learning difficulties described in normal aging and Alzheimer's disease.

Human memory studies with scopolamine also have a relatively long history. Drachman and Leavitt (1974) demonstrated a reversible memory impairment in young normal controls following administration of subcutaneous scopolamine. This deficit, which mimicked the memory impairment of normal aging, could be reversed by the indirect cholinergic agonist, physostigmine, but not with the nonspecific stimulant amphetamine (Drachman 1977). Numerous other investigators have reported similar amnestic effects in normal humans of various ages following administration of scopolamine (Crow and Grove-White 1973; Ghonheim and Mewaldt 1975; Siteram et al. 1978; Caine et al. 1981; Sunderland et al. 1986).

**Table 1. Selected Animal Paradigms Tested with Scopolamine**

| Test Paradigm | Species | N | Scopolamine Dose/Route | Result | Reference |
|---|---|---|---|---|---|
| 1. **Passive Avoidance** | Wistar Rats | 32 | 0.1-1.0 mg/kg IP | Decreased latency across most doses | Itoh et al. (1988) |
| 2. **Go-No-Go Alternating Lever Task** | Sprague Dawley | 7 | 0.125-1.0 mg/kg IP | Decreased accuracy | Heise and Hudson (1985) |
| 3. **2-Way Active Avoidance** | Wistar Rats | 4/group | 20 µg. Intracaudal Cannula | Decreased performance with posterior but not anterior caudate injection | Prado-Acala et al. (1984) |
| 4. **Visual Object Discrimination** | Marmoset Monkeys | 13 | 0.03-0.06 mg/kg IM | Marked improvement at higher doses | Ridley et al. (1984) |
| 5. **Delayed Matching to sample** | Marmoset Monkeys | 8 | 0.03-0.05 mg/kg IM | Decreased aquisition following delay | Bartus et al. (1982) |

**Table 1. Selected Animal Paradigms Tested with Scopolamine (continued)**

| Test Paradigm | Species | N | Scopolamine Dose/Route | Result | Reference |
|---|---|---|---|---|---|
| 6. **Pavlovian Conditioning** | N2 Rabbits | 142 | 20-80 μg/brain area injections | Decreased heart rate after septum and lateral ventricle injections | Powell et al. (1985) |
| 7. **Runaway Habitation and T-Maze Acquisition** | Cockroach | 32 | 500 μg IP | Decrease runaway habitation but no change in T-Maze | Barraco et al. (1984) |
| 8. **Auditory Discrimination Task** | Sprague-Dawley Rats | 8 | 0.006-0.375 mg/kg IP | Linear dose-related decrease in performance | Kirk et al. (1988) |
| 9. **Spatial Learning** | Lister Hooded Rats | 24 | 0.2-0.4 mg/kg IM | Decreased speed rather than accuracy | Willner et al. (1986) |
| 10. **Morris Water Maze and 9-Arm Radial Water Maze** | Hooded Rats | 13-30 | 0.1-1.0 mg/kg IM | Impaired acquistion, memory and navigation | Buresova et al. (1986) |

**Scopolamine Testing in Alzheimer's Disease:**

The pharmacologic model of memory impairment, together with the biochemical evidence supporting an intrinsic cholinergic deficit in Alzheimer brains, encouraged a series of therapeutic trials in Alzheimer's disease with pro-cholinergic agents. As noted earlier, these studies provided only modestly positive results, prompting us to question the functional status of the cholinergic system in Alzheimer's patients (Sunderland et al. 1985). By challenging older normal controls and Alzheimer's patients with various doses of scopolamine, we assessed the relative sensitivity of these two populations to central cholinergic blockade (Sunderland, et al. 1987). The results indicated that not only were Alzheimer's patients still functionally sensitive to the interruption of cholinergic neurotransmission by scopolamine, they were even more sensitive than age-matched controls, suggesting a functional hypersensitivity to scopolamine along behavioral and cognitive, but not physiologic measures. Drachman and colleagues had originally shown that young controls could temporarily demonstrate the memory impairments generally seen in normal elderly subjects after being given a dose of scopolamine (Drachman and Leavitt 1974; Drachman 1977). We extended that work by documenting that normal older controls briefly mimic some of the cognitive patterns of Alzheimer's patients following scopolamine administration (Sunderland et al. 1986).

As for the scopolamine results in Alzheimer patients, there are several immediate implications. First, the cognitive response suggests that viable cholinergic neurons exist in the diseased Alzheimer brain, and that pro-cholinergic agents may still have a role in the treatment of the cognitive impairments. Because of the marked inter-individual variability in pharmacologic sensitivity, however, dose-response issues remain a hurdle and require tedious titration experiments for each patient -- a process that is now common in most current cholinergic trials (Davis and Mohs 1982; Tariot et al. 1987; Newhouse et al. 1988). Second, the behavioral and cognitive hypersensitivity in Alzheimer patients compared to age-matched controls suggests possible differential effects across diagnoses and invites speculation about the diagnostic potential of scopolamine testing. Even if the diagnostic specificity of the scopolamine response is not high for Alzheimer's disease, however, there could also be prognostic implications in the responsiveness of the underlying cholinergic system to scopolamine at various clinical stages of Alzheimer's disease.

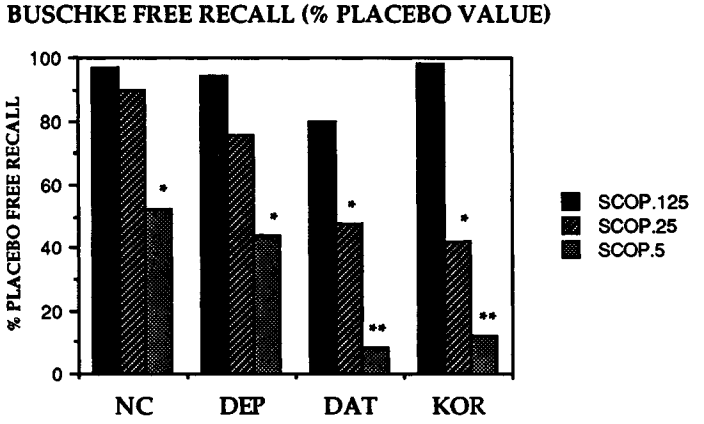
**Scopolamine as a Diagnostic Test:**

To address the issue of diagnostic specificity with scopolamine modelling, we have begun a series of ongoing experiments with non-Alzheimer populations, including elderly depressives (major affective disorder) and Korsakoff's patients (alcohol amnestic disorder). The elderly depressed population was tested first because of the diagnostic confusion which sometimes occurs between Alzheimer's disease and depression in the elderly and because of the numerous areas of biochemical overlap between the two conditions (Sunderland et al. 1985, 1987; Davis et al. 1984, 1988) Any objective diagnostic technique helping to distinguish between dementia and depression would be of great value because the latter is quite treatable.

Korsakoff's patients are usually distinguished from Alzheimer's patients based on their history and presentation, but they too represent a reasonable comparison group because cognitive profiles similar to those with Alzheimer's disease can be seen in Korsakoff's patients (Weingartner et al. 1983; Moss et al. 1986; Mazzucchi et al. 1987).

We have thus far studied nine elderly depressed (69.7 ± 6.1 years) and four Korsakoff patients (63.4 ± 3.1 years) in addition to our original group of Alzheimer patients and age-matched controls. Patients were all diagnosed according to DSM-III-R criteria (APA 1987) and were in good health medically. The test paradigm was equivalent to that previously administered to Alzheimer patients and age-matched controls (Sunderland et al. 1987). Briefly, subjects were drug-free for at least three weeks before receiving a series of scopolamine challenges (0.1, 0.25, and 0.5 mg I.V.), placebo injections, or lorazepam (1.0 mg. P.O.). Drugs were given individually at 9:00 a.m. over five test sessions which were each separated by at least 48 hours. Cognitive testing was administered once a test day approximately 90 minutes after drug and included measures of semantic and episodic memory, vigilance, and attention (for review see Tariot and Weingartner 1986; Sunderland et al. 1987). Behavioral ratings and physiologic measures were obtained hourly for the duration of the test session. Data was analyzed by repeated measures analysis of variance with post hoc comparisons of within-group means accomplished with the Tukey's Honestly Significant Difference Test.

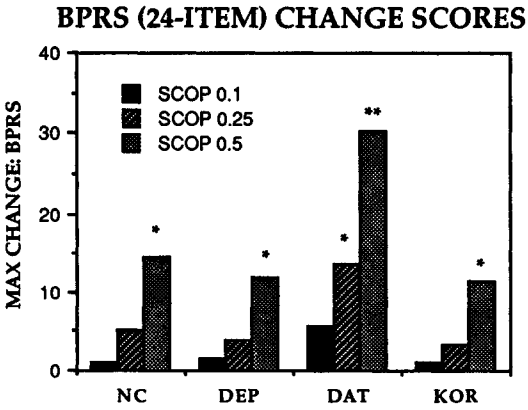
**Figure 1.** Cognitive changes across drug and diagnosis as measured by the free recall portion of the Buschke Selective Reminding Task. Values represent the percentage of placebo scores across drug conditions.




NC=Normal Controls (N=10); DEP=Elderly Depressives (N=9); DAT=Alzheimer Patients (N=10); KOR=Korsakoff's Dementia (N=4); SCOP.125=Scopolamine 0.125 mg IV; SCOP.25=Scopolamine 0.25 mg IV; SCOP.5=Scopolamine 0.5 mg IV; LOR=Lorazepam 1 mg PO; *$p<0.05$; **$p<0.01$

The preliminary cognitive results, as presented in Figure 1, clearly suggest that elderly depressed subjects show a different profile of scopolamine response than Alzheimer patients (Newhouse et al. 1988). Specifically, the depressed patients fail to demonstrate the increased sensitivity to the middle (0.25 mg) dose of scopolamine that is seen with most Alzheimer's patients. In fact, the overall profile of the elderly depressed patients on scopolamine is much closer to that of the age-matched normal controls (Newhouse et al. 1988). On the other hand, the Korsakoff patients appear to show a pattern of cognitive responses more similar to the Alzheimer patients. While the number of experimental subjects in the Korsakoff group is certainly too small to make a definitive statement, the pattern seen in the "free recall" results (Figure 1) is consistent with results previously obtained in Alzheimer patients (Sunderland et al. 1987). Other tests of semantic memory, vigilance, and attention do not demonstrate marked changes at the middle (0.25 mg) scopolamine dose with the Korsakoff patients. Behaviorally, the picture is less clear, because both the depressed elderly and Korsakoff patients show increases only at the highest (0.5 mg) scopolamine dose. This response is similar to the age-matched controls and not the Alzheimer patients, who show an increased behavioral response at both the 0.25 mg and 0.5 mg doses of scopolamine (Figure 2). Thus, the Alzheimer and Korsakoff patients appear to show divergent behavioral patterns following the middle scopolamine dose.

**Figure 2.** Behavioral changes across drug conditions and diagnoses as measured by the 24-item Brief Psychiatric Rating Scale (BPRS). Change scores represent maximum differences from the placebo day.




NC=Normal Controls (N=10); DEP=Elderly Depressives (N=9); DAT=Alzheimer Patients (N=10); KOR=Korsakoff's Dementia (N=4); SCOP.125=Scopolamine 0.125 mg IV; SCOP.25=Scopolamine 0.25 mg IV; SCOP.5=Scopolamine 0.5 mg IV; LOR=Lorazepam 1 mg PO; *$p<0.05$; **$p<0.01$

From these early indications, it would appear that scopolamine testing may be of some help in differentiating demented from non-demented patients (i.e., Alzheimer versus depressed) but that differentiation within the dementias may be less successful, at least with the cognitive data. This impression is of course preliminary, but is further supported by the work of Agid and colleagues who demonstrated that non-demented Parkinson's patients were more sensitive to the cognitive effects of scopolamine than normal controls (Dubois et al. 1987). Further study with more Korsakoff patients, demented Parkinson patients, and perhaps patients with dementia secondary to cerebrovascular accidents should help further clarify these diagnostic questions.

Human Pharmacologic Modelling of Alzheimer's Disease with Scopolamine:

One of the more intriguing findings of the early scopolamine work in humans was that a memory impairment similar to that naturally found in most older subjects could be induced briefly in young controls following scopolamine administration (Drachman and Leavitt 1974). When we later showed that a further dosing of scopolamine in older subjects could briefly mimic parts of the cognitive pattern seen in early Alzheimer's disease (Sunderland et al. 1986), the stage was set for pharmacologically modelling either aging or Alzheimer's disease with scopolamine or other anticholinergic agents. The obvious implication of this modelling approach is that new therapeutic medications could perhaps be screened with this model.

Before proceeding with a review of the animal literature of scopolamine modelling, it should be pointed out that numerous investigators have questioned how completely the scopolamine-induced cognitive impairments mimic the pattern seen in Alzheimer's disease (Beatty et al. 1986; Mohs et al. 1986; Broks et al. 1988; Kopelman and Corn 1988). In addition, researchers have questioned the cognitive mechanisms by which scopolamine has its memory-impairing effect (Callaway et al. 1985; Grober et al. 1989), demonstrated that the pattern of cerebral blood flow following scopolamine in normals is different than the blood flow pattern found in resting, drug-free Alzheimer patients (Honor et al. 1988), and revealed that other non-cholinergic drugs can also produce dementia-like effects (Block et al. 1985). These discrepancies should not be surprising, however, because as was noted earlier, Alzheimer's disease clearly involves more than just the cholinergic system pathologically. Even within the cholinergic system itself, there are still open questions about the relative importance of the muscarinic and nicotinic deficits (Whitehouse et al. 1986; Meyerhoff and Bates 1988; Newhouse et al. 1989). The roles of noradrenergic, serotonergic, dopaminergic, and peptidergic systems in learning and memory are still being actively studied. Nonetheless, the scopolamine challenge model remains the best pharmacologic approximation of memory impairment available today.

The animal literature regarding learning and memory experiments is filled with scopolamine reversal paradigms (see Table 2 for a partial listing). Currently, there is a veritable cottage industry of testing for new pharmacologic agents which will block or reverse the memory impairments induced by scopolamine. In fact, if a

Table 2. Selected Animal Studies of Scopolamine Combined with other Pharmacologic Agents

| Test Paradigm | Species | N | Drug Combination | Results | Reference |
|---|---|---|---|---|---|
| 1. **8-Arm Radial Maze** | Sprague-Dawley Rats | 10 | Scopolamine 0.15 mg/kg + SCH 23390 0.10 mg/kg (or) Raclopride 5 mg/kg | SCH 23390 attenuated scopolamine deficits but not raclopide | Levin et al. (1988) |
| 2. **Passive Avoidance** | Wistar Rats | 6-8/ group | Scopolamine 0.63 mg/kg IP + Oxiracetam 50-100 mg/kg IP (o) Aniracetam 100 mg/kg PO | Partial reversal of scopolamine effects on latency. OXIR also reversed some changes in brain ACh levels | Spignoli et al. (1987) |
| 3. **Amygdala Kindling** | Hooded Rats | 6-10/ group | Scopolamine 10 mg/kg + Naloxone 10 mg/kg | NAL further reduces kindling following scopolamine | Cain et al. (1987) |
| 4. **Delayed Attenuation** | Wistar Rats | 20 | Scopolamine 0.2 mg/kg IP + DN-1417 1-10 mg/kg IP TRH 3-30 mg/kg IP Methamphetamine 0.2-1.0 mg/kg IP | Reduced scopolamine effect following DN-1417 but not TRH or methamphetamine | Yamazaki et al., (1986) |
| 5. **Active Avoidance** | Swiss Webster Mice | 12/ group | Scopolamine 1 mg/kg SQ + Arecoline 0.5-1.0 mg/kg SQ d-Amphetamine 0.5-2.0 mg/kg SQ | Reduced scopolamine effect on latency with arecoline and selected amphetamine doses | Quartermain (1988) |

**Table 2. Selected Animal Studies of Scopolamine combined with other Pharmacologic Agents (continued)**

| Test Paradigm | Species | N | Drug Combination | Results | Reference |
|---|---|---|---|---|---|
| 6. **T-Maze Active Avoidance** | CD-1 Mice | 10/group | Scopolamine 1mg/kg SQ + Fluoxetine 15 mg/kg SQ | Improved retention following fluoxetine plus scopolamine | Flood et al. (1987) |
| 7. **T-Maze Active Avoidance** | CD-1 Mice | 10/group | Scopolamine 1 mg/kg SQ + DHEA-S 162 ng ICV | Reversal of scopolamine deficits when DHEA-S administered | Flood et al. (1988) |
| 8. **Passive Avoidance** | NMRI Mice | 3-8/group | Scopolamine 0.6 mg/kg SQ + WEB 1881 FU 10-100 mg Piracetam 100-500 mg | Attenuation of scopolamine deficits with both agents | Kuhn et al. (1988) |
| 9. **8-Arm Radial Maze** | Wistar Rats | 14 | Scopolamine 0.188-0.750 mg/kg + Baclofen 1.25-2.5 mg/kg | Increased scopolamine errors but reduced hyperactivity in combination | Sidel et al. (1988) |
| 10. **Inhibitory Avoidance Training** | DUB-ICR Mice | 10/group | Scopolamine 3 mg/kg IP + Epinepherine 0.01-0.02 Glucose 10-250 mg/kg Arecoline 0.5-20 mg/kg | Increased latency with selected EPI and GLU doses, but no such attenuation with arecoline doses | Stone et al. (1988) |

**Table 3. Human Studies of Scopolamine Combined with other Pharmacologic Agents**

| Test Paradigms: | N: | Drug Combination: | Results: | Reference: |
|---|---|---|---|---|
| 1. Word List Recall | 10 | Scopolamine 0.35 mg SQ<br>Choline 14 gms PO | No beneficial effect despite doubling of plasma choline level | Mohs et al. (1985) |
| 2. Word List Recall | 13 | Scopolamine 0.43 mg SQ<br>Choline 8 gms PO | Most beneficial effect came with doubling of plasma choline level | Davis and Mohs (1982) |
| 3. Digit Span, Word List Recall & Behavioral Ratings | 20 | Scopolamine 0.43 mg SQ<br>Choline 8 gms PO | Modest positive effect on word list and decrease in tension subscales | Mohs et al. (1981) |
| 4. Rapid Information Processing | 12 | Scopolamine 1.2 mg PO<br>Nicotine 1.5 mg PO | Partial reversal of of scopolamine effect | Wesnes and Revell (1984) |
| 5. Tapping Speed, Digit Symbol, Flicker Fusion & Reaction Times | 14 | Scopolamine 0.3-0.9 mg PO<br>Ephedrine 25 mg PO | Blunted abnormality on Flicker Fusion | Nuotto et al. (1983) |

**Table 3. Human Studies of Scopolamine Combined with Other Pharmacologic Agents (continued).**

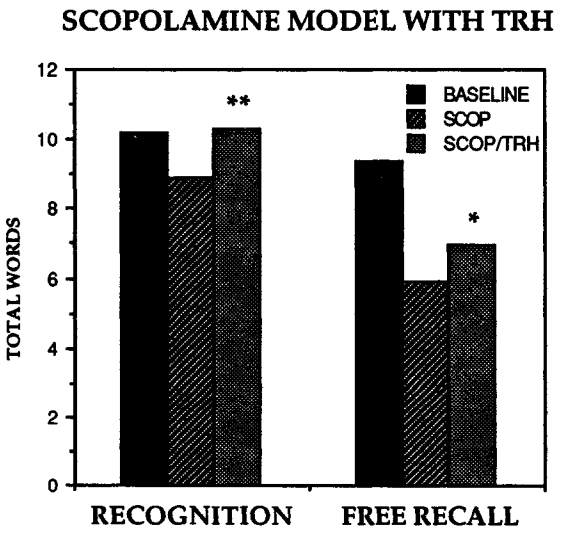
| Test Paradigms: | N: | Drug Combination: | Results: | Reference: |
|---|---|---|---|---|
| 6. Wechsler Memory Subscales | 10/group | Scopolamine 1.0 mg SQ<br>Amphetamine 10 mg PO | Increased alertness and cognitive process on combination | Drachman (1977) |
| 7. Delayed Recall | 10/group | Scopolamine 8 ug/kg IM<br>Methamphetamine 0.2-0.3 mg/kg PO<br>Physostigmine 32 ug/kg IM | Physo and Methamphet both significantly reversed the scopolamine effect | Mewaldt and Ghoneim (1979) |
| 8. Categorized Serial Learning | 14 | Scopolamine 0.5 mg IM<br>Arecoline 6 mg SQ | Arecoline helped reverse slowing of learning found with scopolamine | Siteram et al. (1978) |
| 9. Selective Reminding Category Recall | 12 | Scopolamine 0.5-0.75 mg IV<br>TRH 0.5 mg/kg IV | TRH attenuated SCOP effect in some tests | Molchan et al. (1990) |
| 10. Digit Symbol, Reaction Time & Pattern Memory | 8 | Scopolamine 0.4 mg PO<br>Dextroamphetamine 5 mg PO | No significant benefit with the combination | Schmedtje et al. (1988) |

new drug, irrespective of its postulated mechanism of action, does not at least attenuate the effects of scopolamine, then it may be held suspect as a potential therapeutic agent for Alzheimer's disease.

The scopolamine memory impairment model is much less tested in humans as a screening ground for new agents. Up until now, the focus has usually been whether or not pro-cholinergic agents can, as would be expected, block the anticholinergic effects of scopolamine. Indeed, post-synaptically active cholinergic agents such as arecoline, nicotine, and physostigmine have reversed the majority of scopolamine-induced memory impairments in experimental paradigms with human subjects. Non-specific or less direct agents such as ephedrine, methamphetamine, and the cholinergic precursor choline have been less successful in this human model (see Table 3).

Recently, we have begun a series of experiments testing non-cholinergic agents in the scopolamine model of human memory impairment. Based on our earlier work in animals where the thyrotropin releasing hormone (TRH) analog MK-771 was found to partially block the anticholinergic effects of atropine (Sills et al. 1988), we tested high doses of TRH in humans (Molchan et al. 1990) (Figure 3).

Figure 3. Results of cognitive testing following scopolamine with or without TRH




* P< 0.05, **P<0.01
SCOP = Scopolamine 0.5-0.75 mg IV; SCOP/TRH = Scopolamine 0.5-0.75 mg IV + TRH 0.5 mg/kg IV

The blood brain barrier makes transport of even small peptides such as TRH difficult, so we elected to use large doses (0.5 mg/kg) of this naturally-occuring compound. Even if only 1% of the TRH made it across the blood brain barrier (Metcalf 1982), we estimated that this would be a pharmacologically active dose. The results of experiments where scopolamine administration was followed by high-dose TRH in young normals would support this hypothesis, because the expected scopolamine-induced memory impairment was significantly attenuated by TRH . These findings are encouraging, not only because of the potential therapeutic efficacy of TRH in human memory disorders, but also for the usefulness of the model itself. Further studies of TRH in Alzheimer's disease are now ongoing (Mellow et al. 1989).

**Future Drug Challenge Strategies:**

We have focused primarily on the scopolamine challenge in this chapter, but it is by no means the only approach to pharmacologic modelling in Alzheimer's disease. Even within the cholinergic system, there are other candidate agents for challenges such as physostigmine, the nicotinic antagonist, mecamylamine, and the selective $m_1$ or $m_2$ muscarinic antagonists now under development. Other neurotransmitter systems are also likely sources for more diversified pharmacologic challenges. Within the serotonergic system, for instance, m-chlorophenylpiperazine (m-CPP) has already been administered to Alzheimer patients and elderly controls (Lawlor et al. 1989), but antagonist metergoline remains mostly untested and should provide further information about the serotonergic responsiveness of the aged and diseased brain. Similarly, careful psychopharmacologic investigations of the dopaminergic and noradrenergic systems in older and demented subjects would be of great value. Paradigms employing the neuroleptic haloperidol and adrenergic agents such as clonidine and yohimbine are currently ongoing in several laboratories across the country.

The point of developing new and diverse challenge tests for the study of Alzheimer's disease is two-fold. First, while acetylcholine is clearly a key figure in the cognitive impairment associated with Alzheimer's disease, other systems are also involved, and their degree of impairment needs to be further elucidated. We must continue to expand our understanding of Alzheimer's disease beyond the previously narrow focus on the cholinergic system. Second, the lack of an adequate animal model in Alzheimer's disease research makes it important that we develop human models of reversible memory impairment. Therapeutic options in Alzheimer's disease are currently limited, but as the intense basic science interest in this condition continues to expand and potential new agents are developed, there may soon be an overabundance of potential therapeutic choices for researchers. We need a way to quickly and effectively screen those agents as they become available. The scopolamine model of memory impairment in normal controls may be one such practical approach.

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# Part 6

# Molecular Genetics



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# Biochemical Markers for Alzheimer Disease as Reflectionof the Neuropathology in Cerebrospinal Fluid:

## Application for a Laboratory Diagnostic Test

C. Bancher,* H.M. Wisniewski, P.D. Mehta, K.S. Kim.
I. Grundke-Iqbal and K. Iqbal
*New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY*

Alzheimer's disease (AD) is the most common cause of dementia in modern society, afflicting an estimated three million people, most of them elderly, in the United States alone. AD is an illness of later life; its incidence steeply increases with age, and it develops in up to 15% of individuals older than 65 years of age (Pfeffer et al. 1987). The constant improvement of medical care has resulted in a steady increase in life span and will continue to do so; this increase, in turn, will lead to a disproportionate growth of our elderly population (Rosenwaike et al. 1980). Thus, in the future, the number of AD cases is expected to rise at a quickening pace, rendering this devastating disease an even heavier socioeconomic burden than it already is.

Despite its frequency and its chronicity - the average period between which the first symptoms appear and death occurs is more than 8 years (Barclay et al. 1985) - the diagnosis of AD can at present only be confirmed by a histologic examination of the brain tissue following autopsy or, on rare instances, biopsy. During the patient's lifetime, a positive diagnosis of AD is currently made by psychiatric interview and neurologic examination and can be supported by various psychological tests and brain imaging procedures. By and large, however, the diagnosis is based on the exclusion of other possible causes of dementia. These strategies provide a diagnostic accuracy ranging from 50 to 90% (Eisdorfer and Cohen 1980; Garcia et al. 1981a). The major source of mistake is overdiagnosis, one of the results of the tremendous attention that has been focused on AD during the last several years. A considerable number of patients with a clinical diagnosis of AD actually have other disease processes that mimic AD, some of which are reversible and might be responsive to appropriate therapy. Although AD is not yet treatable, a reliable diagnosis during the patient's lifetime is important to ensure that patients with treatable conditions are not missed. A diagnostic test will help to better identify this population, and also to contribute to

*: C.B. is a visiting scientist from the Neurological Institute, University of Vienna, Austria.

reducing the high costs incurred during the diagnosis of this debilitating disease. In the future, laboratory diagnostic tests may be useful to monitor the efficacy of therapeutic drugs and to detect preclinical cases of AD for early intervention.

## Potential bases for diagnostic tests

Potential bases for diagnostic aids can be sought among the numerous physical parameters that distinguish AD from normal elderly individuals. For example, characteristic patterns of cortical atrophy may be detectable by sophisticated high-resolution neuroimaging techniques (deLeon et al. in press). Specific metabolic abnormalities may be measurable by positron emission tomography (PET) scan and related methods. Abnormal-pattern electroretinograms have been shown to distinghish AD patients from control subjects (Katz et al. 1989; Trick et al. 1989). The familial form of AD appears to be transmitted in an autosomal dominant fashion (Goudsmit et al. 1981), and in some families, an abnormal gene on chromosome 21 (FAD gene) segregates with this disease (St. George-Hyslop et al. 1987). The much more common sporadic form of the disease is also suspected to involve a genetic component (reviewed by Schupf et al. 1989), indicating that specific DNA probes may be employed as potentially useful markers. An infectious etiology of AD has been discussed for a long period of time, and a preliminary report (Manuelidis et al. 1988) has more recently brought up this discussion again. Obviously, a putative infectious agent could represent a biomarker usable in a diagnostic test. However, at present, the transmissibility of AD has yet to be proved, its infectious etiology remains a speculation, and no infectious agent has been discovered.

Another group of approaches is based on several abnormalities that have been observed in various cell types outside the nervous system. For example, the fluidity of platelet membranes has been shown to be increased in the blood of AD patients (Zubenko et al. 1988). The potential of DNA repair after damage induced by alkylation has been demonstrated to be decreased in various peripheral cell types (Bradley et al. 1988) and the repolymerization of microtubules following colchicine treatment appears to be delayed in AD fibroblasts (Matsuyama et al. 1988) and AD lymphoblasts (Krawczun et al. unpublished). A preliminary report has described that fibroblasts cultivated from AD patients but not from controls express antigens associated with paired helical filaments (PHF) (Baker et al. 1988). The cerebrospinal fluid of some AD patients contains antibodies that recognize cholinergic neurons in the rat brain (McRae-Degueurce et al. 1987), and sera of AD patients have been shown to contain antibodies to a neurofilament-like 200-kDa polypeptide present in cholinergic neurons of the electric fish Torpedo (Chapman et al. 1988). Finally, the olfactory epithelium, a tissue relatively accessible for biopsy, appears to display characteristic abnormalities in AD patients (Talamo et al. 1989).

As yet, most of these abnormalities have been observed in relatively small numbers of patients. Since all of them seem to represent peripheral reflections of a primary disease process of the central nervous system, the question arises if these features

may also occur as a result of other underlying causes. Particularly whether these changes are also present in other forms of dementia and other neurodegenerative diseases remains to be determined.

More direct strategies aim at measuring characteristic biochemical abnormalities of the AD brain itself by attempting to detect their traces in body fluids. These biochemical changes can be subdivided into two major categories: (i) those associated directly with the biochemical composition of the chief neuropathologic lesions defining AD, i.e., neurofibrillary tangles (NFT) and neuritic (senile) plaques (NP) and (ii) those apparently unrelated to the proteinaceous compounds found in these lesions. Among the latter are changes in neurotransmitter levels, such as the well known decrease in cholinergic activity (reflected by low levels of AChE (Pope et al. 1964) and ChAT (Davies and Maloney 1976)), decreased levels of the neuropeptide somatostatin (Davies et al. 1980) and imbalances in various other biologically active molecules involved in neural transmission (reviewed by Martin et al. 1988). Some of these abnormalities are reflected in the cerebrospinal fluid (CSF). Most of these changes can be considered consequences of neuronal dysfunction and nerve cell loss, which in turn, are results of the AD neuropathological process affecting the underlying neuronal network.

Biochemical markers that are direct measures of the lesions indicating this pathological process will be the subject of this chapter. For a better understanding of the rationale underlying our approach, we would like to recall a few basic principles of AD neuropathology, the current criteria for the autopsy diagnosis of AD and some of the biochemistry of NFT and NP.

## Neuropathological diagnosis of AD

The brains of AD victims show a diffuse atrophy of the cerebral cortex. Histologically, they are characterized by a more or less severe neuronal loss, gliosis, amyloid angiopathy, granulovacuolar degeneration and the presence of large numbers of NFT and NP. It is actually these two latter lesions that are diagnostic hallmarks of the disease. It is on the basis of the presence of a certain threshold number of NP and NFT that the neuropathologist establishes the diagnosis of AD. Whether these lesions are the only cause of nerve cell dysfunction and death or whether there are other as yet unknown factors affecting neuronal function remains to be determined. However, it is the NFT and NP that are the most intimately associated with the presence of the disease and which actually define today's conception of what AD is: AD is a form of dementia that is histopathologically characterized by the presence of large numbers of NFT and/or NP in the cerebral cortex.

### Neurofibrillary Tangles

At the ultrastructural level, NFT consist of bundles of PHF (Kidd 1963; Wisniewski et al. 1976a). Morphologically and biochemically, PHF are unlike any of the normal neurofibrils (Wisniewski et al. 1984). Their sparing solubility has so far precluded the complete elucidation of their molecular composition; however, several normal cellular components have been shown to be

part of these abnormal fibers. Among the main constituents of PHF are tau (Brion et al. 1985; Grundke-Iqbal et al. 1986a,b; Wischik et al. 1988) and ubiquitin (Mori et al. 1987; Perry et al. 1987).

Tau is a microtubule-associated protein. Its function in normal neurons is to promote the assembly of tubulin subunits into microtubules (Weingarten et al. 1975). In PHF, tau is modified (Grundke-Iqbal et al. 1986b,1988; Nieto et al. 1988). The exact nature and the full extent of the modification are not yet known, but it includes abnormal phosphorylation (Grundke-Iqbal et al. 1986b), possibly at multiple sites (Iqbal et al. 1989a), and other alterations that change tau's biochemical properties and its susceptibility to proteases. It is probably such an altered site of the tau molecule that defines the epitope recognized by the monoclonal antibody Alz-50 (Wolozin et al. 1986; Ksiezak-Reding and Yen 1989).

Ubiquitin is a 76-residue polypeptide involved in the non-lysosomal ATP-dependent degradation of intracellular proteins. Ubiquitin is unusual because it is found (i) as a free molecule and (ii) covalently conjugated to other proteins via a peptide or isopeptide bond between its COOH-terminal residue and an alpha- or epsilon-$NH_2$ group of the target protein, either singly or in branched multiple ubiquitin structures. One of the roles ubiquitin plays is to tag molecules for degradation, making them recognizable as substrates for proteolysis, much as a blaze on a tree serves as a signal for the logger (reviewed by Hershko and Ciechanover 1986). The accumulation of ubiquitin in PHF may represent an unsuccessful attempt of the neuron to eliminate these abnormal proteinaceous structures.

Even though high numbers of NFT can also be found in other diseases, the number of NFT in the cerebral cortex correlates well with the presence and severity of dementia in AD victims (Wilcock and Esiri 1982).

Neuritic (Senile) Plaques

NP are complex structures consisting of several neuronal and non-neuronal elements. Their common denominator are extracellular deposits of amyloid fibers that occur as compact central cores or as tiny wisps. Biochemically, amyloid fibers are different from PHF. Their main proteinaceous component is the beta-peptide (Glenner and Wong 1984). The beta-peptide is a small fragment of a much larger precursor protein, the gene for which resides on the long arm of chromosome 21 (Robakis et al. 1987; Kang et al. 1987). As predicted from *in situ* hybridization studies, levels of this precursor molecule are not elevated in the AD brain (Goedert 1987; Lewis et al. 1988). The amyloid deposits are surrounded by glial cells and by dystrophic degenerating and regenerating neurites some of which may contain PHF (Wisniewski et al. 1983).

The PHF in the plaque neurites are morphologically and biochemically indistinguishable from those forming NFT in the cell body. It has been shown that the neurites in the NP only contain PHF if nerve cells in the same brain area develop NFT (Barcikowska et al. 1989; Probst et al. 1989). It is thus possible to classify NP into two categories: those that contain PHF and those that do not. Globally, the presence of NP does not distinguish demented from non-demented people (Crystal et al. 1988), and the total

number of NP does not correlate well with the severity of dementia (Wilcock and Esiri 1982). Non-demented individuals can have large numbers of NP (Katzman et al. 1988). However, it has been suggested that the subgroup of NP that contains PHF-loaded neurites can distinguish demented from non-demented people (Wolozin and Davies 1987; Dickson et al. 1988). It appears that the presence of this subgroup of NP in the neocortex is actually specific for the presence of AD (Shin et al. 1989; Barcikowska et al. 1989).

Diffuse Neuropil Tangles (Neuropil Threads)

Large quantities of PHF are also found in the so-called neuropil threads, a sometimes very dense network of abnormal nerve cell processes that, in contrast to the NP, are not arranged in clusters (Braak et al. 1986). In large number, these structures also are specific for AD, and it should be emphasized that, in many cases, it is this type of pathology that probably accounts for the majority of the PHF in the AD brain.

## Can a biomarker for PHF be useful?

With all these data taken toghether, it appears that a combination of the three lesions, NFT, NP and neuropil threads (all made of PHF), correlates best with the presence of AD-type dementia. While required for a positive diagnosis, NP alone (and, thus, amyloid) do not correlate with the presence of dementia. AD, therefore, can be defined as a form of dementia, which is characterized by a certain threshold amount of PHF in the cerebral cortex in the presence of NP (Fig. 1).

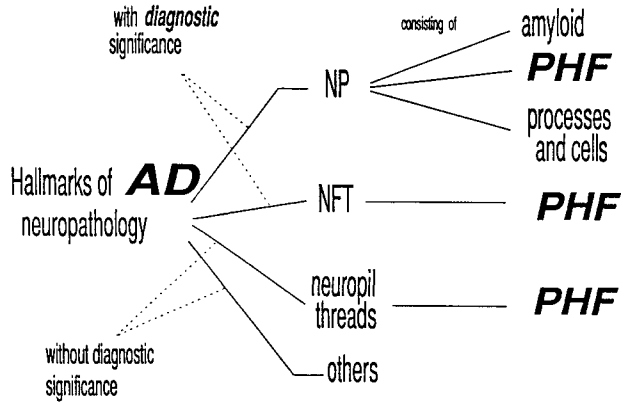



Figure 1. Hallmarks of the neuropathology of AD

PHF, however, are not unique to AD. They may be found in the brains of adult patients with Down syndrome, who invariably develop the neuropathology characteristic for AD (Burger and Vogel 1973). In the form of NFT, PHF occur in many and unrelated diseases, such as Guam Parkinsonism-dementia complex (Hirano et al. 1961), dementia pugilistica (Corsellis et al. 1973; Wisniewski et al. 1976b), postencephalitic Parkinsonism (Hirano and

Zimmermann 1962) and hydrocephalus (Wisniewski et al. 1987a). They have also been described in a few cases of subacute sclerosing panencephalitis (SSPE, measles encephalitis), tuberous sclerosis, lead encephalopathy, lipofuscinosis and Hallervorden-Spatz disease (Wisniewski et al. 1979). Large numbers of NFT can also be found in progressive supranuclear palsy; however, they differ from the NFT of AD by their topographical distribution, their ultrastructure and some of their antigenic properties (Bancher et al. 1987).

Any diagnostic test based on PHF must take the above mentioned conditions into consideration. Fortunately, all of these conditions with PHF can be clinically distinguished from AD. Down syndrome is identified by its characteristic phenotypic expression and karyotyping. Guam Parkinsonism-dementia complex is limited to the natives of the island of Guam (Chamorro Indians). Dementia pugilistica, also called punch drunk syndrome, is an extremely rare disease seen in professional boxers and horse jockeys. Postencephalitic Parkinsonism is rare and is distinguished from AD by its characteristic clinical symptomatology. Hydrocephalus is easily diagnosed with the help of neuroimaging. Lastly, the group of diseases in which only some cases show NFT are rare, occur generally at young age and are clinically distinguishable from AD.

In practice, a biomarker based on the detection of PHF or their traces may thus be helpful for the diagnosis of the disease. We expect it to measure the diagnostic criterion itself, and by this token, it may accurately indicate the presence or absence of the AD degenerative process.

## Monoclonal antibodies to PHF

Preparations of biochemically isolated PHF have been used to immunize mice and to generate hybridomas producing monoclonal antibodies (mAbs) to PHF (Wang et al. 1984). By immunohistochemistry, these mAbs strongly label the NFT, NP and diffuse neuropil tangles (neuropil threads) in AD brain tissue. In cases with severe pathology, most of the staining is accounted for by the presence of a dense network of innumerable neuropil threads. The mAbs also stain isolated NFT, both before and after repeated treatment with boiling SDS. Since mAb 5-25 and 3-39 consistently stain the highest number of NFT, these two mAbs have been selected for further studies. By immune electron microscopy of negatively stained isolated PHF, it has been confirmed that the immunoreactivity resides on the PHF themselves and that it is not sensitive to treatment of the sample with a proteolytic enzyme (Merz et al. 1986; Wrzolek et al. 1987). The fact that the immunoreactivity is not abolished even after harsh treatments with detergents and proteases suggests that the mAbs are indeed directed against polypeptides tightly associated with the PHF and not against non-specific proteins trapped in the NFT or adhering to the PHF. On Western blots of isolated PHF, both mAbs label PHF polypeptides with molecular weights between 45 and 62 kDa (Grundke-Iqbal et al. 1985), similarly to those seen with rabbit antisera to PHF and with antibodies to tau (Grundke-Iqbal et al. 1986a,b). However, they do not react with tau from normal human brain or the abnormally phosphorylated tau present in unpolymerized form in the cytosol fraction of AD brain.

It should be mentioned at this point that on histological sections the PHF mAbs also react with varying intensity with several other elements in the tissue, including granular material in the white matter, corpora amylacea, the media of blood vessels, Lewy bodies (Galloway et al. 1988; Bancher et al. 1989a) and nuclear chromatin. The labelling of nuclear chromatin is reminiscent of what one observes with antibodies to ubiquitin, probably because of the ubiquitin conjugates with histones $H_2A$ and $H_2B$ (Goldknopf and Busch 1977; West and Bonner 1980). This observation has led to studies on the reactivity of the PHF mAbs with ubiquitin itself. Both 5-25 and 3-39 label ubiquitin on Western blots. Immunoassays with fragments of ubiquitin have revealed that 5-25 reacts with amino acid residues 64-76, i.e., the COOH-terminal end region of ubiquitin, whereas 3-39 reacts with residues 50-65 (Perry et al. 1989). However, the reactivity of the mAbs has been found to be greater with isolated PHF than with free ubiquitin on dot blots (Grundke-Iqbal et al. 1988). These data indicate that the epitopes recognized by the mAbs to PHF may be defined by the specific state ubiquitin assumes if conjugated to its target protein in PHF. Several lines of evidence support this possibility. Many antibodies raised to artificial ubiquitin conjugates react with NFT only if the tissue has been denatured by formalin fixation and paraffin embedding, whereas antibodies recognizing ubiquitin but raised to PHF do not require such denaturation (Perry et al. 1989). Conversely, mAb 5-25 recognizes PHF in biochemically isolated NFT and on frozen tissue sections, but not in paraffin-embedded tissue. These findings indicate that antibodies generated against PHF recognize the ubiquitin conjugate found in PHF as is, whereas antibodies raised to artificial ubiquitin conjugates in general do not (Perry et al. 1989).

The selective recognition of ubiquitin conjugates by different mAbs has been noted previously. Similar to our PHF antibodies, MEL-14, a mAb raised to lymphocyte homing receptor, recognizes an epitope defined by the union of ubiquitin with the receptor (St. John et al. 1986). On the ubiquitin sequence, the MEL-14 epitope is contained within the same residues as those recognized by 5-25. The reactivity of this mAb with free ubiquitin is weak but can be increased by denaturation of the sample with SDS.

All these data together suggest that the conjugation of ubiquitin to different target proteins leads to specific effects on the tertiary structure of the ubiquitin polypeptide. These conformational alterations are expressed as distinct epitopes recognizable by well-defined mAbs. The portion of ubiquitin most likely to be affected is the COOH-terminal region, the site for conjugation and thus the portion nearest to the carrier sequences. It is this part of the ubiquitin molecule that is recognized by our mAbs to PHF (Perry et al. 1989). Whether the epitope recognized by mAb 5-25 resides on a specific conformational state of ubiquitin specifically found in PHF or whether it represents the junction site of the COOH-terminus of ubiquitin with its target protein in the conjugate remains to be determined. The latter possibility is favored by the fact that both mAb 5-25 and 3-39, but not antibodies generated to artificial ubiquitin conjugates, recognize the same bands on immunoblots prepared from PHF fractions as those stained by antibodies to tau; yet, neither

5-25 nor 3-39 recognize tau prepared from human or bovine brain (Grundke-Iqbal 1985, 1986a,b, 1988). These findings indicate that the mAbs may recognize epitopes formed by ubiquitinated tau.

The mAbs to PHF thus recognize PHF-specific altered sites on a normal cellular protein. This point is crucial since all proteinaceous constituents of PHF so far discovered are also present in normal tissue. In consequence, it must be expected that antibodies raised to PHF will react with normal proteins. This is confirmed by the reactivity of antibodies raised against PHF with normal tau and ubiquitin (Delacourte and Defossez 1986; Ihara et al. 1986; Kosik et al. 1986; Grundke-Iqbal et al. 1988; Mori et al. 1987; Perry et al. 1989).

It should be noted in this context that A68, a polypeptide recognized by the mAb Alz-50, has been claimed to be a protein unique to AD (Wolozin et al. 1986). However, Alz-50 labels PHF (Love et al. 1989) and it has recently been demonstrated that A68 is an abnormal form of tau (Ksiezak-Reding and Yen 1989). This modified form of tau appears to be specific to neurofibrillary pathology and could thus be useful as a marker for the presence of PHF. Indeed, A68 has been detected in the CSF of AD patients but appears to be absent from the CSF of non-demented subjects (Wolozin and Davies 1987). These data were obtained from a relatively small number of patients and their validity remains to be confirmed. Alz-50 also reacts strongly with tau from normal human brain (Ksiezak-Reding et al. 1988a,b). However, the specificity problem arising from this cross-reactivity may possibly be overcome by the use of certain test conditions. Alternatively, antibodies with higher specificity for A68, i.e., antibodies that are able to differentiate modified tau in PHF from normal tau may prove useful (Minger et al. 1989). The same applies to two recently described abnormal tau species (tau 64 and tau 69) present in AD brain, which are recognized by antisera to tau and PHF (Flament and Delacourte 1989). Again, these abnormal molecules may well be reliable markers for neurofibrillary degeneration, but the antisera used for their detection also react strongly with normal tau.

Whether the reactivity of our mAbs to PHF with several structures not containing PHF on histological sections of normal and AD brain tissue is a reflection of the presence of other ubiquitinated proteins remains to be seen. Whether and to what degree this cross-reactivity will blur reactivity arising from PHF in a given test system can only be determined by post-mortem studies of the brains of tested patients.

## A competitive inhibition ELISA for PHF

Our approach for developing a diagnostic test system for AD is based on the possible detection of unpolymerized, soluble PHF polypeptides in the CSF. Western blots of CSF from AD patients immunostained with mAb 5-25 revealed several large polypeptides, but only trace amounts of reactivity in the region of the molecular weight of free ubiquitin (Iqbal et al. 1989b). In contrast, an antiserum raised to ubiquitin that readily detects 10 ng of ubiquitin on the blot did not detect any immunoreactive polypeptides, and especially no free ubiquitin in the CSF. These

data indicate that the CSF does not contain more than trace amounts of free ubiquitin and that most of the immunoreactivity of CSF with mAb 5-25 is not due to the presence of free ubiquitin.

We used a competitive inhibition enzyme-linked immunosorbent assay (ELISA) with mAb 5-25 to quantitate levels of PHF immunoreactivity in the CSF of patients with AD and in controls. The theoretical principles of the assay are shown in Figure 2. The competitive assay includes a preincubation step in which the unlabelled antibody and the antigen (patient CSF) are first allowed to react with each other (step 2). This reaction mixture is then placed in a microtiter well that has been precoated with antigen specific for the antibody (step 3). In the preincubated antigen-antibody mixture, the fewer the antibody molecules that remain available, the more PHF antigen that is present in the CSF. In other words, if high levels of PHF antigen are in the CSF, much antibody will be absorbed, and little will be left to bind to the antigen-coated plate (left). Conversely, if lower levels are present, more antibody will bind (right). After an incubation period, an enzyme-labelled antiserum directed against the primary antibody is added and allowed to bind. The addition of a chromogen enables the reaction to be visualized and quantitated (Mehta et al. 1985) (step 4). The more antigen that is present in the CSF, the less intense the reaction that takes place; the less antigen that is present, the more intense the reaction. In interpreting the test, measurement of the optical density (OD) correlates with the intensity of the reaction. Thus, a low OD indicates a positive test (left), and a high OD, a negative test (right).

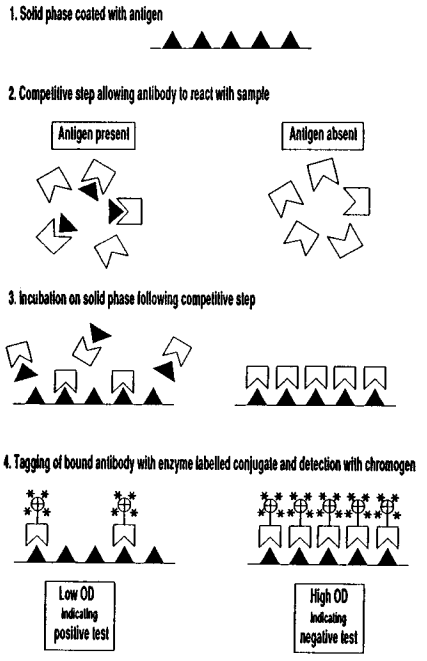



Figure 2. Principle of competitive inhibition ELISA

## PHF antigen in the cerebrospinal fluid

In our initial experiments, we examined the CSF from nine patients with clinically diagnosed AD (mean age ± SD: 64 ± 10 years) and nine non-AD controls (mean age ± SD: 57 ± 19 years). The non-AD controls consisted of patients with stroke, seizures, multiple sclerosis and other neurological conditions. Only AD patients meeting research diagnostic criteria for the diagnosis of AD (Eisdorfer and Cohen 1980; McKhann et al. 1984) were included. The specimens were coded before the experiments. The mean level of PHF antigen in patients with AD was significantly greater than that of other neurological patients ($p < 0.05$, one-tailed t test), although there was considerable overlap. Eight of the nine AD CSF specimens contained more than 0.2 units of PHF antigen, whereas only three of nine controls exceeded this level (Mehta et al. 1985) (Fig. 3).

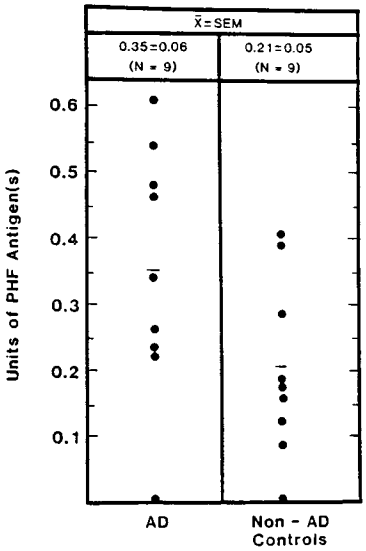



Figure 3. Concentration of PHF antigen in the CSF of AD patients and controls. (from: Mehta et al. 1985)

In another experiment, 40 CSF specimens were obtained from 14 patients with AD (mean age ± SD: 63 ± 8 years) and 26 non-AD controls (mean age ± SD: 51 ± 18 years). The latter included patients with multiple sclerosis, seizures, migraine, vasculitis, stroke and other neurological conditions. When a modified competitive inhibition ELISA was used, the CSF from the AD patients showed a higher degree of inhibition of binding between the PHF-coated plate and mAb 5-25 (mean ± SD: 72.6 ± 30.6) than did the CSF from the controls (mean ± SD: 47.9 ± 21.8). This difference was statistically significant ($p < 0.005$, one-tailed t test). Again, however, some degree of overlap was observed between the two groups (Wisniewski et al. 1989a) (Fig. 4).

Since the neuropathology of AD is also characterized by the presence of extracellular amyloid deposits, we examined the CSF for the presence of both PHF and beta-peptide immunoreactivity. Coded CSF samples from 19 patients included five patients with AD

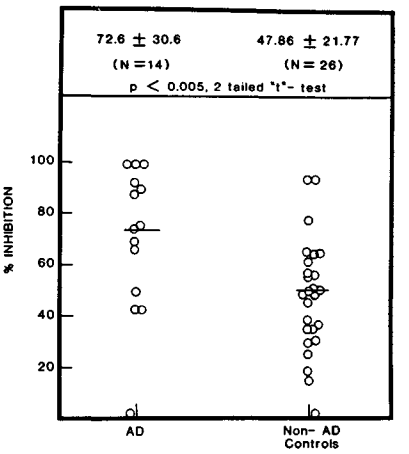



Figure 4. Percent inhibition of PHF antibody binding after preincubation with CSF from AD patients and controls. (from: Wisniewski et al. 1989a)

(mean age ± SD: 74 ± 6 years) in late stages of the disease and 14 non-AD controls (mean age ± SD: 66 ± 13 years). The controls included patients with multi-infarct dementia, unspecified dementia and CNS tumor. PHF antigen was quantitated by a two-step competitive inhibition ELISA using mAb 5-25 and ubiquitin as the standard. When the code was broken, CSF from the AD patients had significantly higher ($p < 0.025$) reactivity than that from the controls (Fig. 5).

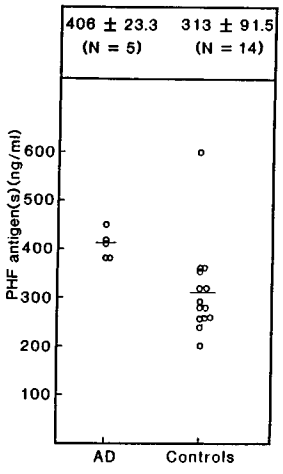



Figure 5. Concentration of PHF antigen in the CSF of AD patients and controls. (from: Wisniewski et al. 1989a)

Beta-peptide antigen in the CSF

To assay for beta-peptide antigen, we used a mAb (4G8, $IgG_{2b}$) raised to a synthetic peptide corresponding to the first 24 amino acids of the beta-peptide. By ELISA, it has been shown that the epitope recognized by this antibody resides on residues 17-24 (Kim et al. 1988). On Western blots, this mAb does not react with ubiquitin or tau. In total brain homogenate, it recognizes a doublet of approximately 125 kDa, most likely corresponding to the amyloid precursor protein, and several bands of lower molecular weights that may represent the precursor at different stages of catabolism (Grundke-Iqbal et al. 1989). In the tissue, this antibody stains vascular and plaque amyloid and neuronal lipofuscin but does not react with NFT or NP neurites (Grundke-Iqbal et al. 1989; Bancher et al. 1989b). It also stains the lipofuscin-like storage material in different forms of neuronal ceroid lipofuscinosis (Wisniewski et al. 1989). In the AD brain, it reacts with beta-amyloid deposits in various morphological forms, including typical plaques and large areas of diffuse amyloid infiltration in a neuropil by and large free of dystrophic neurites (Wisniewski et al. 1989b). In contrast to antibodies to antigens associated with PHF, it also stains all the NP found in the brains of non-demented elderly people (Barcikowska et al. 1989).

The above mAb was employed to quantitate beta-peptide immunoreactivity in the CSF of the same 19 patients that had been tested with mAb 5-25 to PHF. Beta-peptide antigen was detected in both the AD patients and the controls. However, the concentrations did not differ significantly between the two groups (Fig. 6). This result is not surprising for the following two reasons. (i) Since the beta-peptide is a fragment of a large precursor molecule, this precursor as well as several of its non-amyloidogenic fragments carries the same epitopes as the

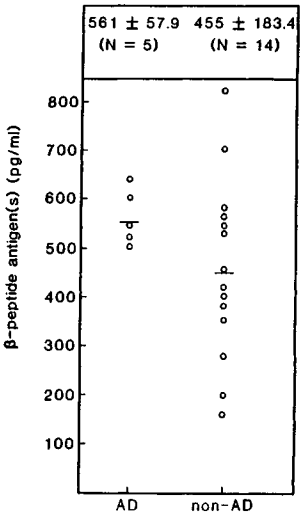



Figure 6. Concentration of beta-peptide antigen in the CSF of AD patients and controls. (from: Wisniewski et al. 1989a)

beta-peptide and must be expected to react with antibodies raised against this sequence. The amyloid precursor appears to be a normal cellular protein and to have soluble derivatives in the CSF (Weidemann et al. 1989; Palmert et al. 1989; Ghiso et al. 1989). Cross-reactivity arising from these molecules will interfere with reactivity of possible beta-peptide in the CSF, and levels of immunoreactivity may not reflect the amount of amyloid present in the brain. (ii) The total number of NP, and thus the amount of amyloid in the brain, does not correlate well with the presence of dementia and cannot be used as a diagnostic criterion for AD. Non-demented aged individuals as well as aged patients with non-AD dementia but high NP counts may represent false positive samples. However, since the deposition of amyloid has been shown to precede the appearance of PHF in Down syndrome (Wisniewski et al. 1987b; Mann and Esiri 1989), a similar time course must be suspected for AD, and it cannot be ruled out that non-demented individuals with high numbers of NP but no or few PHF are at a preclinical stage of AD. Whether such patients may be identified by high levels of beta-peptide antigens in their CSF is at present unknown.

## Interpretation

We have shown that CSF from clinically diagnosed AD patients has a higher concentration of PHF antigen than does CSF from non-AD controls of comparable age with several other dementing and non-dementing neurological diseases. The mechanism by which polypeptides associated with PHF may make their way from the intraneuronal compartment into the CSF is at present obscure; however, our results and those of others (Wolozin and Davies 1987) indicate that such a phenomenon takes place. Whether this mechanism requires the degeneration of the PHF-bearing neuron is also unknown. The recently shown presence of the amyloid P-component, a serum protein, in NFT (Duong et al. 1989) indicates that even large molecules can cross the blood-brain barrier and the cell membrane of affected neurons. The permeability of the neuronal plasma membrane for macromolecules under pathological conditions has been demonstrated previously (Garcia et al. 1981b; Lossinsky et al. 1987). Measuring PHF antigen in the CSF may actually mean measuring the total number of PHF in the brain (in NFT, NP and neuropil threads), much as the neuropathologist establishes the diagnosis of AD. Brain biopsy or autopsy verification by histopathology on the cases studied is not yet available but will be needed to assess the validity of the test system. Histopathology may also help explain the overlap between PHF antigen levels of the AD patients and the controls.

False positive results may be due to increased presence of cross-reactive elements in the brain tissue. Nothing is known on the solubility properties of these structures, but for theoretical purposes the existence of soluble derivatives in the CSF has to be considered. Especially corpora amylacea can be numerous in the aging brain and are preferentially found near the pial and ventricular surfaces. Theoretically, increased numbers of Lewy bodies could also lead to a false positive test since these lesions contain ubiquitin (Kuzuhara et al. 1988) and react with the PHF mAbs (Galloway et al. 1988; Bancher et al. 1989a). Diffuse Lewy body disease, characterized by the widespread occurrence of Lewy bodies in the cerebral cortex, has recently

been shown to be a significant substrate of dementia in old age (Byrne et al. 1989; Dickson et al. 1989) and represents an important differential diagnosis to AD. However, as seen by immunocytochemistry with antibodies to ubiquitin or PHF, the total amount of reactivity in the tissue of these cases is only a small fraction of what one observes in the AD brain. This difference makes it unlikely that high numbers of Lewy bodies will be reflected by elevated levels of 5-25 immunoreactivity in the CSF.

In addition to NFT and Lewy bodies, ubiquitin has been identified in several other filamentous intracellular inclusions, including Pick bodies, Rosenthal fibers, Mallory bodies, Lafora bodies and others (Manetto et al. 1988, 1989). Again, these lesions are unlikely to interfere with the test system since they either (i) do not occur in conditions to be considered as differential diagnoses to AD or (ii) do not produce an amount of immunoreactivity in the tissue comparable to that accounted for by NFT, NP and diffuse neuropil tangles in AD.

## Detection of preclinical cases?

However, positive results in non-demented cases in the test system could also represent individuals with greater than normal levels of neurofibrillary changes. These individuals could be in the "incubation time" of AD, a period of time in which the brain is still able to compensate for the damage arising from the degenerative process. It is conceivable that some of these patients are at a preclinical stage of AD in which a certain number of lesions are already present but can still be accommodated. At this stage, symptoms of dementia are not yet clinically apparent.

In this context, it is interesting to note that neither the number of NFT nor the number of NP increases during the last years of the disease, whereas the number of cortical pyramidal neurons shows a significant decrease until death (Mann et al. 1988). In other words, if disease activity is reflected by the number of "active" neuropathological lesions (i.e., PHF in neurofibrillary changes and deposits of amyloid), it may reach a steady state and may not necessarily increase during the late stage of the disease. If these data can be extrapolated to earlier disease stages, extensive PHF formation may occur relatively early on and could lead to positive results in the test system before dementia is clinically detectable.

The early diagnosis of such cases will be important for therapeutic intervention before a significant loss of the neuronal tissue has occurred. Neurons do not divide, and irreversibly damaged or lost circuits cannot be expected to regenerate. However, if the active disease process is detected at an early stage, an appropriate therapy may arrest the degenerative process before it has had time to destroy an amount of neurons, processes and synapses that can not be compensated for.

False negative results in the test system might represent AD cases with borderline histopathology. Alternatively, they may be due to clinically misdiagnosed cases of dementia that actually have other underlying diseases. The correlation with autopsy

results will clarify these issues.

## A diagnostic test based on PHF is feasible and useful

The correlation of PHF immunoreactivity in the CSF with the clinical diagnosis of AD suggests that a laboratory diagnostic test based on this assay may be feasible. Although the range of values overlap between clinically diagnosed AD patients and individuals with other neurologic diseases, the higher values may serve as a useful adjunct to the diagnosis when combined with appropriate clinical and neuropsychological findings. Screening of large numbers of cases, especially with neuropathological confirmation at multiple medical centers must be carried out to evaluate the specificity and sensitivity of the test. Given the complex nature of the pathology, it is likely that no single biochemical marker will allow a certain diagnosis of AD. Therefore, it is important that multiple approaches be developed to design diagnostic aids for different forms of dementive diseases. A laboratory test measuring the amount of PHF present in the brain will be a valuable tool for the differential diagnosis of dementia in the elderly population.

## Summary

In neuropathology, the most important feature determining the diagnosis of Alzheimer dementia is the presence of paired helical filaments (PHF) in neurofibrillary tangles and neuritic (senile) plaques. A monoclonal antibody was raised to a preparation of biochemically isolated PHF. This antibody recognizes a PHF-specific epitope defined by the union of the carboxy terminal end of ubiquitin with its target protein in PHF, possibly tau. Based on the hypothesis that antigens associated with PHF quantitatively transfer into the cerebrospinal fluid (CSF), we used this antibody to evaluate the CSF of Alzheimer patients and controls. The CSF of Alzheimer cases had significantly higher levels of PHF antigen than that of the controls, even though the range of values overlapped between the two groups. Levels of beta-protein antigen did not significantly differ between Alzheimer patients and controls. The reasons why the results of the test system are not always in agreement with the clinical diagnosis remain to be determined. However, a laboratory diagnostic test based on the detection of PHF antigen in the CSF is feasible and may help to detect both clinical and preclinical cases of Alzheimer disease.

## Acknowledgements

We thank Dr. M. Elzinga for critically reading the manuscript and Ms. M. Stoddard Marlow for editing the English syntax. Mr. L. Black provided invaluable help with the bibliography. The figures were prepared under the photographic supervision of Mr. R. Weed. We appreciate the expert and patient secretarial assistance of Ms. J. Kay. Supported in part by grants AG0-4220 and HD-22634 from the National Institutes of Health.

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# Immunochemical Approaches to the Diagnosis of Alzheimer Disease

Benjamin Wolozin
*Laboratory of Clinical Science*
*National Institute of Mental Health, Bethseda, MD*

Alzheimer disease (AD) produces a profound alteration of the chemistry and structure of the central nervous system (CNS). These changes leave behind them distinct biochemical footprints. Each footprint offers the possibility of producing a diagnostic test, providing that a method of detection that is both specific and sensitive can be developed. The development of immunologic methods for biochemical detection, in the form of polyclonal antisera and monoclonal antibodies, has given scientists the ability to create such probes.

The field of Alzheimer disease (AD) research has seen a proliferation of relevant antibodies and relevant antigens to study, as well as new methods with which these studies can be carried out. With each advance has come the specter of a potential diagnostic test. Yet while many likely targets for diagnostic tests have been identified, and antibodies for detecting these targets created, the development of a routine, clinically useful test for AD has proven elusive.

The following chapter will review the current status of immunochemical detection of Alzheimer disease. I will begin by describing the basics of immunochemical detection: the terminology used in immunology and the methods used in antibody generation and detection. The focus of the chapter will then switch to a review of the antibodies that have been developed, the antigens detected and their relevance to the development of clinically useful tests. The benefits and drawbacks of these potential tests will be discussed. Finally, future directions for immunochemical detection of AD will be outlined.

## A PRIMER ON IMMUNOLOGY

Clinical tests relying on immunochemical detection of molecules have become commonplace in the medical world. The pregnancy test found relies on the use of antibodies to detect the presence of ß-HCG in urine or blood. Tests for HIV exposure detect the presence of anti-HIV antibodies in the serum of those at risk for the disease. Tests for Systemic Lupus Erythematosus often include examining for the presence of anti-nuclear antibodies. The critical factors in all of these tests have been the ease of acquiring specimens, the recognition of target molecules and the development of detection methods that are both sensitive and specific (Harlow 1988). A diagram of sample immune reactions is shown in figure 1A.

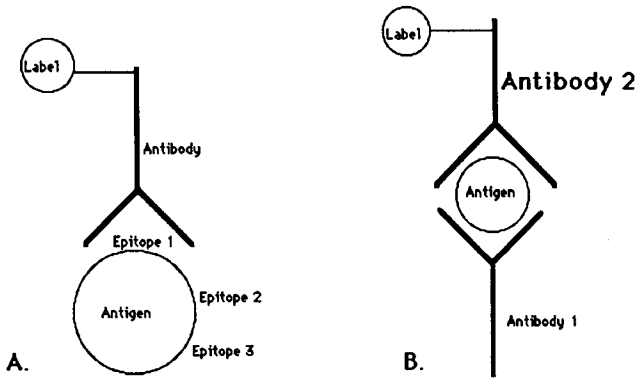



Fig. 1. Schematics of immune complexes. A. A labelled antibody binding to an antigen with multiple epitopes. B. A sandwich hybridization assay.

Every immunologic test requires a target molecule, which the antibody will detect. This target molecule, such as ß-hCG in the pregnancy test, is termed the **antigen**. Often the antigen is a fairly large molecule such as a protein and the antibody only binds to one part of protein. The site on a molecule recognized by the antibody is termed an **epitope**. A large molecule such as a protein has many potential epitopes.

Antibodies themselves come in many different forms. Antibody molecules can be grouped into several different classes: IgG, IgM, IgA and IgE (where "Ig" stands for immunoglobulin). Antibodies used in clinical tests are generally of the IgG or IgM class. Antibodies are always made by inoculating animals. The serum from these animals can be obtained and

used directly. Because the animal's immune system generates many antibodies after any given inoculation, the subsequent serum contains many different antibodies which can bind to the multiple epitopes present on the antigen. This serum is referred to as a **polyclonal antiserum**, or if the antibodies have been purified from the serum, a **polyclonal antibody**.

Individual antibodies can be isolated from an immunized animal by a method relying on transforming individual antibody producing cells from that animal so that they can proliferate indefinitely. Then the cells are diluted so that colonies of cells can be grown from individual transformed antibody producing cells. Any individual lymphocytes only produces one particular type of antibody, therefore, since these colonies are monoclonal in origin, each monoclonal colony produces only one particular type of antibody. The subsequent antibodies obtained from these monoclonal colonies are termed **monoclonal antibodies** (Kohler 1975). Every antibody within a solution containing a particular monoclonal antibody is identical. Consequently, a given monoclonal antibody solution will only recognize one epitope on a antigen.

Two basic approaches exist for isolating the antibody - antigen complex. One approach, termed an **Enzyme Linked Immunosorbant Assay (ELISA)**, is to fix the antigen to a solid phase (such as the bottom of a plastic dish) so the complex won't wash away, and to detect the antigen with a specific antibody labelled in a manner to make it detectable. Unbound antibody is simply washed away. Alternatively, the antigen can be labelled, often with radioactivity. Then a known amount of antigen is added to the specimen solution containing an unknown amount of antigen. Unlabelled antibody solution is allowed to react with the mixture of labelled and unlabelled antigen. Unbound antigen can be adsorbed away with an agent such as charcoal, and the amount of remaining label quantitated; because unlabelled antigen competes for binding with labelled antigen, more bound label correlates with less antigen in the specimen solution. This type of assay is referred to as a **Radio-immuno Assay (RIA)** (Yalow 1959).

Two slightly more sophisticated methods exist for antibody - antigen detection. The sensitivity of an ELISA assay can be increased using a **Sandwich Hybridization Assay** (figure 1B). This method requires the use of at least two different monoclonal antibodies. The first antibody, antibody-1 is bound to the solid phase. The sample solution is then allowed to incubate with this antibody bound solid phase; the antibody preferentially adsorbs the target antigen. The solution is washed away,

and the second antibody, labelled with a detectable marker, is allowed to bind to the antibody-1-antigen complex, giving an antibody1-antigen-antibody2 complex. This results in increased sensitivity. Information regarding physical characteristics, such as the molecular weight of the antigen, can be obtained using the **Western Blot** method. In this method the antigen is subjected to polyacrylamide gel electrophoresis and the contents of the gel are bound to the solid phase. The position of the antigen on the gel indicates its molecular weight. This method is useful in weeding out cross-reactive antigens that have different physical characteristics (being different in size for instance). The Western blot technique has proven particularly useful in confirming the results of positive HIV tests (Murphy 1989).

The methods of detection utilized in these assays fall into three categories: radioactivity, enzyme amplification and, more recently, scintillation proximity detection. Radioactivity can be counted directly. The scintillation proximity assay can also be counted directly. A conformationally sensitive ligand is attached to the antibody. Binding of the antigen alters the antibody resulting in the ligand giving off photons which can be detected by a scintillation solution. Alternatively, a functional enzyme, such as peroxidase or ß-galactosidase, can be attached to the antibody. Incubation of the enzyme with a particular substrate produces a colored reaction product that can be visualized; for instance incubation of peroxidase with 2,2-diaminobenzidine produces a brown reaction product.

Sample acquisition for the above tests relies on urine or blood specimens, both of which can be acquired by relatively non-invasive means. AD, being a disease of the CNS, is not expressed peripherally, although this assumption has come into question, as will be discussed below. Consequently, the CNS must be accessed to obtain an appropriate specimen for analysis. Sampling cerebrospinal fluid (CSF), using a lumbar puncture is the method of choice, however this procedure is much more invasive than giving blood or urine, the amount of specimen that can be obtained is limiting, and the amount of any particular antigen in the CSF is often quite low (Harrington 1988). Hence, any test for AD faces large hurdles to overcome.

## TESTS BASED ON PAIRED HELICAL FILAMENTS

As mentioned, the prerequisite for the development of an immunochemical test for AD are the generation of an antibody or antiserum that recognizes an antigen that is specific to AD, and the

detection of that antigen in some accessible manner, such as in CSF. Initial studies on AD focused on use of the neurofibrillary tangle as an antigen. Selkoe and others showed that an antiserum could be generated that recognize epitopes specific to neurofibrillary tangles (NFT) (Ihara 1983). This antiserum recognized a specific component of the neurofibrillary tangle the paired helical filament (PHF). If an antigen exists in brain parenchyma then it is likely to be present in CSF, though often in far lower concentrations. Wizniewski, Iqbal and their team used this philosophy to guide some generated against PHF's and developed an assay to detect the immunoreactivity in CSF (Mehta 1985). As shown in figure 2, they were able to detect PHF related immunoreactivity in CSF. There was much greater reactivity in CSF from patients with AD than in patients who were not demented. However, there is a significant amount of overlap, as much as 25%, between the two comparison groups, indicating that the test, as designed, is not yet clinically useful. Thal and Davies, using monoclonal antibodies, found a similarly high degree of sample overlap (Thal 1987).

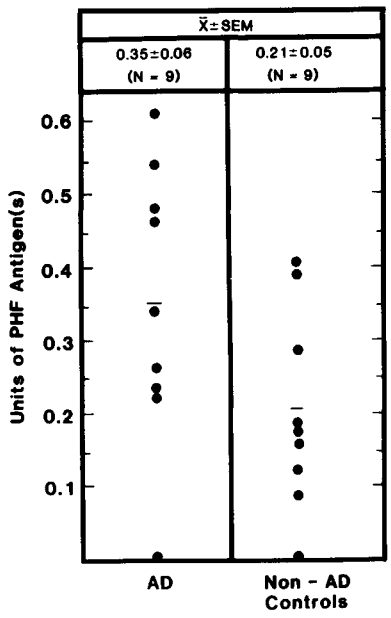



Figure 2. Relative concentration of PHF antigens in CSF of patients and a group of non-AD controls (Mehta1985).

In developing any test one should expect a certain amount of overlap due to clinical uncertainty in the diagnosis of the target group. The frequency of incorrect diagnosis is steadily diminishing due to the use of more sophisticated clinical measures, as is discussed in previous chapters. However, many of the earlier cerebrospinal fluid based studies did not have the benefit of such a carefully analyzed patient population. Without use of these more precise clinical measures, up to 30% of the patients could be incorrectly diagnosed as having AD, and will have other types of dementia such as multi-infarct dementia, Pick's disease or depression

(Sulkava 1983, Fuld 1986). Similarly, some of the group used as the normal comparison group may have AD that is not clinically detectable. Though 15% of the population is at risk for AD, it is not clear how large a fraction of a normal control proband should be expected to exhibit biochemical manifestations of AD without clinical symptomatology. Individuals could manifest initial stages of Alzheimer pathology prior to the onset of clinical symptomatology. In addition, neurofibrillary tangles can be found in low numbers in the brain of cognitively intact elderly individuals at the time of death, and neuritic plaques can exist in high numbers in the brains of nondemented individuals (Tomlinson 1970, Katzman 1988). Whether this represents presymptomatic Alzheimer's disease is unclear .

Most of the overlap in an ELISA based test such as the PHF based test is due to the biochemical inadequacies of the test rather than difficulties in correctly defining the test proband. As will be discussed in the CSF based work with the monoclonal antibody Alz-50, when detection of low level antigens is attempted a significant amount of binding to unrelated antigens often occurs. This produces a high percentage of false positives. Many of the false positives in the PHF based CSF test described above probably stem from such problems and do not relate to the amount of pathology in the brains of those individuals tested.

## STUDIES WITH NEUROFILAMENTS

Further analysis of antibodies that recognize PHF's revealed that some of these antibodies cross-reacted with neurofilament molecules (Anderton 1982, Ishii 1979). Neurofilaments come in three forms that are defined by their molecular weight: 180 (NF-H), 160 (NF-M) and 70 KD (NF-L). These proteins are among the most abundant proteins in the CNS. Their function, while not completely understood, appears to relate to maintaining the caliber of large axons. The neurofilament - neurofibrillary tangle cross reactive antibodies recognized a particular portion of the neurofilament molecule, the C terminal multiphosphorylation domain (Lee V. 1988, Sternberger 1985). This highly phosphorylated region is present on the high and middle weight neurofilament molecules. The involvement of the phosphorylated epitopes in the neurofibrillary tangle suggests that phosphorylation state may be a critical element in its development.

Neurofilaments are phosphorylated when they enter the axon. Hence, all neurofilaments are phosphorylated. This prevents the use of antibodies that react both with PHF epitopes and neurofilament molecules as diagnostic tools. These antibodies are useful for immunocytochemistry,

though, because the density of phospho-neurofilament epitopes present in the NFT makes NFT containing neurons stain much darker than unaffected neurons (Goldman 1986). In this sense, these antibodies are similar to a Bielschowsky stain (silver stain) that can detect NFT's based on the aggregation of neurofilaments (Escourolle 1978). AD brains do contain approximately two fold more phospho-neurofilament reactivity compared to the brains of unafflicted individuals, as judged by immunochemistry (Wolozin 1986a). However, these antibodies are of no value for diagnostic testing because the baseline level of reactivity present in neurologically normal individuals is too high.

## TAU PROTEIN

The recognition of NFT's by anti-neurofilament antibodies may actually represent not binding to neurofilament proteins, but rather cross-reactive binding to a microtubule binding protein, termed Tau protein (Ksiezak-Reding 1987). This protein is present in high abundance in the NFT and contains a highly phosphorylated region similar to that of neurofilaments (Lee G. 1988). With the exception of one antibody, all anti-neurofilament antibodies that recognize the NFT also recognize Tau protein (Gambetti 1986).

Tau is a protein that copurifies with tubulin during repeated cycles of polymerization and facilitates tubulin polymerization (Cleveland 1977). It is found exclusively in axons of CNS neurons (Lewis 1989). Tau protein increases in abundance steadily during development peaking neonatally and then decreases in abundance afterwards (Kosik 1989c).

In the normal brain tau proteins vary in molecular weight from 55 - 65 KD. The reasons for this heterogeneity arise from differential processing of tau mRNA molecules. Tau protein are the products of two genes, one gene is expressed in higher abundance during gestation, the other is expressed in higher abundance during adult life (Kosik 1989c, Goedert 1989). The products of the genes are then each processed to yield up to four different mRNA transcripts (Lee G. 1988). Each of these transcripts is translated and then translationally modified. The result is the presence of 4-6 (and perhaps more) biochemically distinct Tau proteins, depending on the particular tissue analyzed (Cleveland 1977). Hence, Tau proteins exist as a family of proteins.

Tau proteins isolated from the AD brain range in molecular weight from 68 - 55 KD (Grundke-Iqbal 1986a,b). One reason for the slower electrophoretic mobility of Tau from AD brains may be that in AD tau is

phosphorylated (Grundke-Iqbal 1986a,b). The appearance of multiply phosphorylated tau is particular to the process of neurofibrillary tangle formation. High molecular weight Tau proteins are not detectable in the normal brain, although they may also occur during the process of heat shock (Papasozomenos 1989, Julien 1982).

The presence of altered Tau Proteins as a result of the pathology of Alzheimer Disease bodes well for the development of immunochemically based diagnostic tests for AD in that it should be possible to create antibodies that react epitopes unique to the high molecular weight tau species. Unlike neurofilament phospho-epitopes, which are present in normal brain tissue, the high molecular weight tau epitopes should be able to differentiate AD brain tissue from normal brain tissue.

Most antibodies to tau protein are not specific to this NFT epitope though. For instance, Tau 2 and 5E2 are two monoclonal antibodies that recognize all forms of tau including those present in normal brain (Binder 1985, Kosik 1988). Most tau, even that in the AD brain, exists in the 55 - 65 KD forms and so the total amount of Tau is not altered during the pathologic processes of AD. Hence, Tau 2 and 5E2 cannot distinguish AD from normal brain on the basis of simple quantitative tests. Similarly, antibodies that recognize tau, NFT and neurofilaments show too much binding to normal brain to be of quantitative value for the diagnosis of AD (the reasons for this are described above in the section on neurofilaments).

One antibody has been generated that is sensitive to the phosphorylation state of tau is Tau 1, an antibody generated by Binder (Binder 1985). Tau 1 discriminates between tau from AD and normal brain very well using brain homogenates, although the binding is specific to the normal form of tau rather than specific to AD pathology. ELISA assays on brain homogenates show 30 fold greater binding to tau from normal brain than that from AD brain (Wolozin 1986a). Hence, a decrease in Tau 1 reactivity correlates with the presence of neurofibrillary pathology. Tau 2, though, has not been tested on CSF. A CSF based test with this antibody might yield a high number of false positives because the loss of reactivity in any given CSF sample could represent either a positive test or inadequate sensitivity for tau.

ALZ-50 AND A68

One of the antibodies with the highest potential for the development of a test for AD is the monoclonal antibody Alz-50 (Wolozin 1986b). As will be discussed below, the binding characteristics of Alz-50 are

complimentary to Tau 1, though the identity of the antigen for Alz-50, termed A68, remains unclear. Alz-50 recognizes an antigen that has a size range of 60 - 68 KD in Alzheimer brain. The amount of reactivity in normal brain is at most 2% that of AD brain (Wolozin 1987). The reactivity that is present in normal brain corresponds to an antigen whose size range is 60 - 66 KD. Abundant evidence suggests that this antigen may be Tau protein (Nukina 1988, Ksiezak-Reding 1988a,b). Alz-50 recognizes tau protein and the size range of the antigen recognized by Alz-50 is quite similar to that of tau protein. Like AD related tau epitopes, the Alz-50 epitope appears to be phosphorylation sensitive in that treatment with acid phosphatase reduces the amount of reactivity by 50% (Kosik 1989a). On the other hand, since cross reactive epitopes appear to occur among cytoskeletal proteins the recognition of tau by Alz-50 suggests the identity of the antigen but does not prove it (Kosik 1989b). Purified A68 appears to have biochemical properties somewhat different than that of tau; for instance, A68 does not remain soluble in acid solution and A68 appears to form aggregates more readily than Tau. Do these differences represent meaningful biochemical differences, or is A68 a subset of Tau proteins with particular biochemical properties?




Figure 3. Detection of A68 by Western blot analysis in CSF from presumptive AD patients (A,B) and nondemented patients (C). The doublet of 68 KD seen strongly in A and weakly in B is characteristic of A68 staining in CSF.

Whatever the identity of the antigen, it is clear that Alz-50 is an early indicator of neurofibrillary pathology. Neurons can be found that are Alz-50 reactive yet show no NFT reactivity as indicated by histochemical markers such as thioflavine S (Wolozin 1986b, Hyman 1987).Kosik has suggested

that Alz-50 recognizes an epitope that becomes available as tau or some other microtubule binding protein loses its functional binding ability, thereby uncovering a particular epitope.

The ability of Alz-50 to identify an antigen abundant in AD brains and virtually absent in those of normals makes this antibody an excellent candidate for the creation of an AD related diagnostic test. As with the PHF related tests, a positive signal with the antibody should indicate the presence of NFT pathology. The simplest tests with Alz-50 focus on the detection of A68 in CSF using ELISA methodology. Unfortunately, very little antigen is presentin CSF necessitating the concentration of the proteins present in CSF. As the amount of protein present in the ELISA assay increases the chance for nonspecific binding increases. ELISA tests with Alz-50 done in this matter show no correlation of reactivity with disease. However, when the CSF is examined using the Western blot methodology, the reason for this lack of correlation is clear. Using the high protein concentrations necessitated in order to get reactivity, the amount of non-A68 related reactivity that occurs can be greater than that of the A68 related binding in any given patient (figure 3). Use of the western blot methodology allow discrimination between these types of binding and, using the western blot method, the correlation of A68 concentrations with disease state is quite high (Wolozin 1987). However, such a test is not yet clinically useful because the western blot method is relatively cumbersome, and the result obtained is visually complicated.

One solution to this problem has been to develop other antibodies to A68. One of these antibodies, termed PHF-1, shows interesting binding properties in that it recognizes A68 but shows much less binding to tau than does Alz-50 (Greenberg 1989). PHF-1 is also of interest because its sensitivity for A68 is much greater than that of Alz-50. An antibody like PHF-1 might be able to be used in a sandwich hybridization assay along with Alz-50. With this assay, PHF-1 could be attached to a solid substrate. The CSF could then be incubated with PHF-1 and the antigen in it effectively concentrated. Though PHF-1, like any antibody, would have some nonspecific reactivity, its pattern is likely to be different than that of Alz-50, because it recognizes a different epitope. Hence a much more specific and sensitive test could be generated; the only antigen that both antibodies recognize would be A68. Such a test is in fact in development, though no data is yet available (Davies 1989).

None of the antibodies mentioned so far are disease specific. All of these antibodies appear to recognize antigens present in all neurofibrillary tangle including those in Pick;s disease, progressive supranuclear palsy and

Guam-Parkinson's dementia complex (Wolozin 1987). Hence, the reactivity of the antibodies is technically NFT related reactivity rather than AD related reactivity. It turns out that this relatively broad pathologic specificity does not decrease the potential specificity of the antibody tests for AD. The reason lies in quantitative considerations of the amount of pathology. AD brains have 30 times as much Alz-50 reactivity per a gram wet weight as do other diseases with NFT pathology. The reason for this most likely lies with the extensive neuritic involvement in AD that is absent in other diseases. The potential disease specificity of these tests is yet greater because of spacial considerations. AD affects practically the entire mass of the brain, whereas Pick's disease affects only the frontal and temporal cortex and PSP affects only the brain stem. If CSF reactivity were related only to the mass of affected tissue then the amount of reactivity present in AD brains should be at least several times greater than that of other diseases with NFT pathology, independent of considerations related to the density of antigen present.

AMYLOID

Amyloid is the material that accumulates in the center of neuritic plaques. The term, amyloid, is a generic pathology term not specific to AD. Multiple different types of amyloid exist (Frangione 1989). The constitution of amyloid depends on the disease process, however the process of AD produces a specific amyloid peptide.

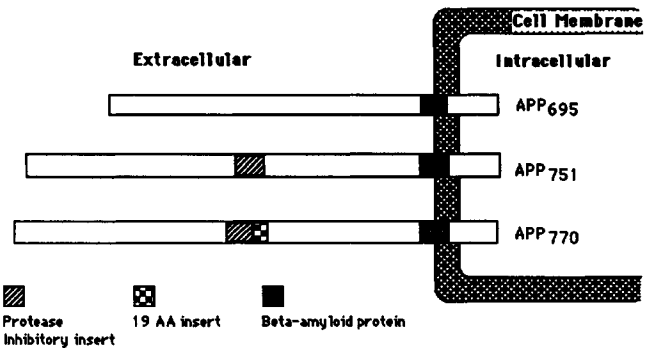



Figure 4. Structure of the three forms of the amyloid precursor proteins, based on the model proposed by Kang (1987). This figure was adapted from Palmert (1989).

The molecular biology of amyloid is covered in detail in earlier chapters and will be only briefly reviewed now in order to provide

background information for assessing the potential role of immunochemical detection of amyloid in the diagnosis of AD. The amyloid peptide found in the brains of AD patients consists of a 42 residue peptide that is precipitated in ß-pleated sheet formation in the center of neuritic plaques and in cerebral blood vessels (Wong 1985). In 1984 this protein was sequenced, and, using this information the gene for the precursor protein was discovered (Glenner 1984, Kang 1987, Goldgaber 1987). As was discussed in the chapter on molecular biological approaches to AD, with the discovery of the gene for the amyloid precursor protein (APP) and the localization of the amyloid gene to chromosome 21, efforts focused on the use of probes for this gene as potential diagnostic markers for AD (Goldgaber 1987). Though this effort failed, the studies of APP may yet yield useful diagnostically relevant information (Van Broeckhoven 1987, Tanzi 1987). The processing of the APP gene product has proved to be very interesting. The APP gene product is spliced to yield 3 different mRNA species, and these are translated to yield 3 APP species containing 695, 751 and 770 residues (figure 4) (Kang 1987, Ponte 1988, Tanzi 1988, Kitaguchi 1988). The difference between the 3 species is the presence of an insert, 56 and 75 residuesrespectively, that functions as a protease inhibitor. The inhibitory action of APP may function in promoting neuronal survival and/or growth (Whitson 1989, Alstiel 1989). This inhibitory sequence has been described in other proteins and is termed a kunitz inhibitor. Thus, APP695 is not a protease inhibitor, APP751 and 770 are inhibitory. APP 770, in fact appears to be identical to a previously sequenced blood protease inhibitor termed Nexin II (Oltersdorf 1989, Van Nostrand 1989). All cells, neuronal and somatic, central and peripheral, have all forms of APP though the ratio of the various forms differs. CNS neurons have a roughly equal abundance of 695 to 751 and 770 (Neve 1988, Johnson 1988). Somatic cells have a higher prevalence of the protease inhibitory forms of APP (Weidermann 1989). Though the biological targets of the protease inhibitory functions of APP are unknown, it does appear that blockade of the kunitz inhibitor in APP is deleterious to cell growth.

The relative abundance of the three forms of APP may be altered in the course of AD, though the direction of change is a point of controversy (Johnson 1988, Palmert 1988). However, what is clear is that APP is not broken down in the normal brain to yield appreciable amounts of the 42 residue peptide that appears in neuritic plaques (Palmert 1988). This suggests that there may be a processing enzyme involved in the pathophysiology of AD that cleaves APP to yield the amyloid peptide. APP like immunoreactivity has also been detected in NFT's (Hyman 1989). Thus, such a processing enzyme might also be relevant to NFT formation.

The search for such a processing enzyme is currently an area of intense investigation.

The relevance of detection of APP and the amyloid peptide have been studied using both CSF and peripheral tissues. APP can be detected in CSF (Weidermann 1989, Palmert 1989). The levels of APP, though, are not altered by the disease process. The reason for this most likely derives from the broad distribution of APP which is present in both affected and unaffected areas. As a result, quantitation of APP does not appear to be relevant to the diagnosis of AD at this time. One might expect quantitation of the amyloid peptide to correlate more closely with the AD disease process. Using immunochemical methodologies, amyloid can be detected in CSF. However, at this point in time no statistically significant differences have been detected between the levels in CSF of patients with AD and age matched controls (Wisniewski 1989). Though improved detection methods and larger sample sizes may yield statistically significant elevations in amyloid associated with AD, the presence of amyloid in the brains of individuals who do not have AD will certainly hinder the application of a CSF based amyloid test towards the diagnosis of AD (Katzman 1988).

## COMING OUT OF THE CLOSET

A principal stumbling block in the immunochemical diagnosis of AD is the detection barriers put up by the CNS: the difficulty of obtaining CNS biopsies and the low level of antigens present in CSF. The ability to detect AD using peripheral markers for the disease would have a large impact on the method of diagnosis of AD. Many investigators are currently studying such approaches.

One approach is to find less invasive methods of obtaining CNS biopsies. Talamo and colleagues have searched for AD pathology in olfactory epithelium (Talamo 1989). They find that these neurons do show early signs of AD including increased immunoreactivity to neurofilaments, as well as the presence of Alz-50 and anti-tau immunoreactivity. Olfactory neurons can be accessed using only local anaesthesia. Though the biopsy procedure is quite delicate, the procedure is simpler and less risky than cortical biopsies.

Another approach is to detect peripheral manifestations of AD. Recent evidence suggests that detection of amyloid in peripheral tissues could be useful as a diagnostic test. Skin biopsies were obtained from 10 patients with AD and 23 patients who were not demented or had dementia's other than AD (Joachim 1989). 9/10 patients with AD showed

faint, perivascular anti-ß amyloid immunoreactivity, while 4/23 controls showed such immunoreactivity. The immunoreactivity was faint and the identity was not confirmed by other methods such as the Western blot. However, if true these data suggest that skin biopsies may be useful in the diagnosis of AD. These results are also interesting because they strengthen the case that AD is not strictly a disease of the CNS. Multiple studies have documented alterations in the metabolism of somatic cells in patients with AD (Baker 1988, Peterson 1986). AD may be a disease affecting the entire body but whose principle manifestation is neurologic.

Tau protein may also be able to be detected in peripheral tissues. Binder has been able to detect tau like immunoreactivity in the nuclei of macrophages (Loomis 1989). Tau appears to be localized in the nucleolar organizing region (involved in the synthesis of ribosomes). An intriguing aspect of this finding is that tau is found in association with chromosome 21. As with peripheral detection of amyloid, the results have yet to be confirmed by Western blot analysis. One possible source of cross-reactivity are the highly phosphorylated nuclear proteins, such as histones, which bear antigenic homology to tau proteins (Ksiezak-Reding 1988). None-the-less, the finding is potentially quite interesting and important. Perhaps, this tau protein will also show AD specific changes. If so, diagnosis of AD might require only a blood test.

Whether these exciting recent findings will stand the test of time and prove useful for the diagnosis of AD remains a matter for conjecture. In addition to the recent findings mentioned above there are many other important areas of research in AD not covered in this review because they are not directly relevant to the diagnosis of AD at this time. There are two particularly interesting subjects that the reader may desire to investigate. One subject is the involvement of ubiquitin, a ubiquitous protein (hence its name) that targets proteins for degradation, which is implicated in the formation of neurofibrillary tangles (Mori 1987). A second subject is the discovery of the presence of antichymotrypsin inhibitor in neuritic plaques (Abraham 1988). This research in particular is likely to spawn much future research studying protease involvement in AD. If an "AD specific" protease is found, it may be of both diagnostic value and a potential target for therapeutic agents.

## SUMMARY

Each AD related antigen that has been detected and found to be specific to the disease is a potential candidate for immunochemical diagnosis of the disease. Several candidate tests already exist. One test is

based on PHF like immunoreactivity. A second test is based on detection of AD related changes in tau protein and a third test is based on detection of the AD related antigen A68. All of these tests suffer from the need to use CSF as the test sample, the low quantities of antigen present in the CSF, and the consequent methodologic difficulties involved therein. Some exciting new prospects for diagnosis do exist. Though preliminary, recent evidence suggests that specific manifestations of AD may exist and be detectable peripherally, thus making specimen acquisition much easier.

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# Part 7

# Brain Biopsy



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# Anatomical Criteria for the Biopsy Diagnosis of Alzheimer Disease

Margaret M. Esiri
*Departments of Neuropathology and Clinical Neurology*
*Radcliffe Infirmary, Oxford, U.K.*

## INTRODUCTION

Diagnostic brain biopsies are not often performed in cases of progressive dementia. This is despite the commonest cause of the condition, Alzheimer's disease (AD), requiring microscopic examination of the brain for confirmation of the diagnosis. There seem to be four principal reasons for the sparing use of cerebral biopsy for the diagnosis of AD. First, there has until now been no specific, well-founded treatment for AD, and an invasive procedure such as a brain biopsy is considered by most clinicians to be unjustifiable for the diagnosis of an untreatable condition in an elderly person. Second, several causes (though not all) of progressive dementia other than AD can be diagnosed, at least with a high degree of accuracy, on the basis of clinical presentation and less invasive investigations such as scanning procedures and cerebrospinal fluid examination. Third, there is an understandable reluctance to risk inadvertent contamination of neurosurgical equipment by the transmissible agent of Creutzfeldt-Jakob disease, a rare cause of rapidly progressive dementia, with the danger of iatrogenic transmission of this disease. Finally, biopsy series reported in the literature show that in a large minority of cases no specific diagnostic features are found, leaving the diagnosis still in doubt.

Nevertheless, in some circumstances, and particularly in those aged less than 65 years, some clinicians consider that management can be improved if a definitive diagnosis is reached relatively early in the course of the disease, and some relatives may press for an answer. In such circumstances a biopsy may be performed. If treatment for AD is developed that is effective but not cheap or trouble-free the demand for a biopsy diagnosis of AD may increase, unless a specific test of a less invasive type is developed in the meantime. As far as research purposes are concerned, a confidence level for the clinical diagnosis of AD of around 80% or more has been attained in some reported series (Miller and Schwartz 1978; Molsa et al. 1985; Sulkava et al. 1985; Schrenberg et al. 1987; Berg et al. 1988). This may be

adequate for some purposes, but it is insufficient for others. For example, genetic studies of possible familial cases require as firm a diagnosis of AD as possible, and in some circumstances it is important to exclude this diagnosis with equal certainty. Moreover, it should be noted that some clinico-pathological studies have recorded a much lower accuracy of clinical diagnosis (Todorov et al. 1975; O'Connor et al. 1988; Homer et al. 1988). Histological confirmation of a clinical diagnosis of AD has been described in 90% of cases in some series in which autopsy examination was eventually carried out (Terry and Katzman 1983; Joachim et al. 1988). However, the fact that the pathologist is aware of the clinical diagnosis may have a bearing on these results. Furthermore, regular performance of brain biopsy cannot expect to match such a high success rate. The small size and the limited acceptable sources of a cortical biopsy, coupled with the fact that it is likely to have been taken relatively early in the course of the disease, when decisions about management have still to be taken, mean that a neuropathological diagnosis is harder to achieve than when the patient eventually succumbs and dies. In addition, although AD appears to have been relatively confidently diagnosed or excluded in cases in the small biopsy series so far reported, the diagnosis has usually been made in subjects under the age of 70years. If biopsies were to be undertaken in an older age group it is likely that the diagnosis would be harder to make, since more overlap of the pathological changes with those of normal ageing has been remarked on in several autopsy studies of AD in the very elderly (Tomlinson and Corsellis 1984; Kemper 1984; Mann et al. 1984, 1987; Ulrich 1985; Katzman et al. 1988; Zubenko et al. 1989).

## PUBLISHED CRITERIA FOR THE NEUROPATHOLOGICAL DIAGNOSIS OF AD.

In most of the studies reporting on the use of cerebral biopsy for the diagnosis of AD, some of which are listed in Table 1, this diagnosis was based on the presence of both senile plaques (SP) and neurofibrillary tangles (NFT) in the sample of neocortex removed.

Table 1. Some published studies of cerebral biopsy for the diagnosis of dementia.

| Reference | No. of cases | % diagnosed as AD | No diagnosis |
|---|---|---|---|
| Smith et al. 1966 | 59 | 57 | 28 |
| Coblentz et al. 1973 | 15 | 66 | 6.5 |
| Torack 1978 | 29 | 38 | 45 |
| Neary et al. 1986 | 24 | 75 | 25 |
| Martin et al. | 11 | 100 | - |

This criterion accords well with what were, until recently, widely applied criteria for the diagnosis of AD at autopsy: the presence of many SP and NFT in neocortex and hippocampus and plentiful granulo-vacuolar degeneration in the hippocampus. Most, but not all, cases also show amyloid deposition in small leptomeningeal and cortical blood vessels and the presence of Hirano bodies in the hippocampus (Tomlinson and Corsellis 1984). The difficulty of accepting these qualitative criteria for the diagnosis of AD became clear when quantitative studies were performed on cases of mentally preserved elderly subjects, substantial numbers of whose brains were shown to contain considerable numbers of SP and NFT (Blessed et al. 1968; Tomlinson et al. 1968,1970; Dayan 1970; Ball 1976, 1978;Ulrich and Stahelin 1984). Nevertheless, distinction between the demented and the mentally preserved with respect to SP and NFT in the brain remains relatively easy in those aged under 60 or 65 years, an age at which a biopsy may be contemplated for the investigation of progressive dementia, since at this age the numbers of NFT and SP in the neocortex are very low except in cases of AD. Thus, most neuropathologists would consider it reasonable, in a subject below the age of 65, to make a diagnosis of AD if both SP and NFT were present in the biospy. What is harder to say is whether such a diagnosis can be made if only a few SP and no NFT are present; it is also uncertain if a diagnosis of AD can be excluded if these features are not present. Follow-up studies of cases that have shown no pathology in a cerebral biopsy would be helpful in clarifying this point, but unfortunately very few such studies are available. One report (Torack 1979) suggested that the prognosis, at least for survival, is better if the biopsy is normal, perhaps because such cases may have included some subjects who were depressed rather than demented. Another point that adds to the uncertainty in interpreting the significance of the biopsy findings is the fact that both AD and another cause of dementia can, and not infrequently do, co-exist in the same patient (Tomlinson et al. 1970; Kurucz et al. 1981; Schoenberg et al. 1987; Wade et al. 1987). For example, the diagnosis of AD on the basis of biopsy appearances does not rule out the possibility that there may be co-existing cerebrovascular disease, even though this may not be evident in the biopsy. This particular combination of pathologies can often be clinically diagnosed if the possibility is thought of.

Above the age of 65 or 70 it may be expected that some subjects not suffering from AD, but developing dementia for some other reason, may harbour at least a few SP in the neocortex. Furthermore, some studies have shown a considerable proportion, around 30%, of those that are demented, but show no other pathology than AD at autopsy, have only SP and no NFT in the neocortex, though almost all such cases have NFT in the hippocampus or parahippocampal gyrus (Terry et al. 1987; Joachim et al. 1988 ). At this age, therefore, any criterion that can be expected to provide a basis for diagnosing AD in a biopsy that lacks NFT needs to be based on a quantitative estimate of the numbers of SP present. Unfortunately, we do not have widely agreed quantitative criteria for distinguishing between AD and the changes of normal ageing, particularly at a relatively early

stage in the development of dementia.

Progress is, however, now being made in developing such criteria for the autopsy diagnosis of AD, where the number of cases available for study is large. Even in this situation, however, difficulties become apparent the more carefully it is studied. Ideally, we need prospective studies of large numbers of demented and non-demented elderly subjects who have been serially tested for mental abilities during life and who have had a comprehensive neuropathological examination of the brain after death. Such studies are difficult and expensive to mount, need to continue for many years and require, on the neuropathological side, application of widely agreed, optimal techniques for studying AD-related pathology. In fact at present, we have to make do with the results of several relatively small studies. These are, for the most part, retrospective, though prospective ones are beginning to be reported now. Most reports include very few prospectively assessed mentally normal subjects as controls, and they use differing criteria for diagnosing dementia during life and differing methods of studying the brain after death (Blessed et al. 1968; Tomlinson et al. 1970; Dayan 1970; Tomlinson and Henderson 1976; Ball 1976, 1977, 1988; Wilcock and Esiri 1982; Tierney et al. 1988; Koster et al. 1989).

A useful starting point for the establishment of agreed neuropathological criteria for the diagnosis of AD is the publication of Khatchachurian (1985) which lists minimal microscopic criteria necessary to establish a post mortem histological diagnosis of AD (Table 2).

Table 2. Summary of the Khatchachurian (1985) recommendations for the neuropathological diagnosis of AD at autopsy.

1) Minimum areas to be sampled:
 3 regions of neocortex (frontal, temporal, parietal)
 amygdala
 hippocampal formation
 basal ganglia
 substantia nigra
 cerebellar cortex
 spinal cord

2) To diagnose pure AD exclude other causes of organic dementia and find in any microscopic field of area 1 sq.mm.:
 In patients less than 50 years:
  more than 2-5 SP and NFT
 In patients between 50 and 65 years:
  more than 7 SP + presence/absence of NFT
 In patients between 66 and 75 years:
  more than 10 SP + presence/absence of NFT
 In patients older than 75 years:
  more than 15 SP + presence/absence of NFT

EXCEPT THAT

3)in the presence of a positive clinical history of AD these criteria should be revised downwards to an extent that needs to be determined by further research. (One suggestion was that only 50% as many lesions need be found in neocortex to retain a high degree of confidence in the diagnosis).

4)Recommended methods: 5-15u paraffin sections stained by Bielschowsky, thioflavin S or Congo red. Each laboratory to test the degree of sensitivity of the methods before deciding on a method of choice.

These criteria were drawn up by a panel of distinguished North American neuropathologists. Several recent autopsy studies have applied them, some with considerable success (Joachim et al. 1988; Zubenko et al. 1989), though others have found them less satisfactory (Crystal et al. 1988; Lemy et al. 1990). A recent paper (Tierney et al. 1988) compared 9 different pathological criteria for their ability to confirm a careful clinical diagnosis of AD (or its absence). Whereas the Khatchachurian criteria specify the number of plaques that need to be present in the neocortex for a diagnosis of AD to be made, these authors found the highest agreement with the clinical diagnosis of AD was achieved by examining the hippocampus. They recommended that a diagnosis of AD be made if SP and NFT each reached a threshold average number of 2 or more per mm 2 on scanning the entire pyramidal cell layer of the hippocampus in an 8u section (Lewis et al. 1988). This criterion has the disadvantage that it could not, of course, be adapted to apply to biopsies since these are almost invariably taken from frontal or temporal neocortex. Ball et al. (1988) also advocate the use of criteria based on examination of the hippocampus to diagnose AD at autopsy.

## SHORTCOMINGS OF PUBLISHED CRITERIA FOR THE NEUROPATHOLOGICAL DIAGNOSIS OF AD

One of the likely sources of disparity between different histological studies that apply the neuropathological criteria suggested to diagnose AD is the variety of possible techniques that may be used to stain SP and NFT. Some recent reports have compared different traditional stains or compared traditional stains with immunostaining for this purpose(Table 3).These studies have generally shown that certain stains (e.g.Bodian, Gallyas, Cross' modification of the Palmgren) selectively stain NFT, but are poor for showing SP. Other stains (e.g.thioflavine S, Congo red, methenamine silver) show SP particularly well, but show NFT poorly (Figure).

Table 3. Comparison of staining techniques for SP and NFT

| Ref | Stains compared | Feature(s) studied | Results |
|---|---|---|---|
| Lamy et al. 1990 | Beil, Cross Gallyas N-F Bodian MS Thio S | SP and NFT | Cross, Bodian and Gallyas preferentially stained NFT. Biel gave best results for both SP and NFT. MS and Thio S preferentially stained SP. N-M insensitive for SP and NFT. |
| Wisniewski et al. 1989 | Biel Bodian Thio S Immunoreaction for beta amyloid protein | SP | Best demonstration of SP obtained with Biel or immunostain for beta amyloid protein after formic acid pretreatment. |
| Yamamoto and Hirano 1986 | Biel Bodian Thio S | NFT | 15-75% more NFT seen with Biel than Bodian stain. Biel probably also showed more NFT than Thio S. |
| Yamaguchi et al. 1988 | Biel Bodian Congo red Immunoreaction for beta amyloid protein | SP | Best demonstration of SP obtained with Biel or immunostain for beta amyloid protein after formic acid pretreatment. |

Biel = modified Bielschowsky stain
N-M = Naumenko-Feigin stain
MS = Methenamine silver stain
Thio S = Thioflavin S stain

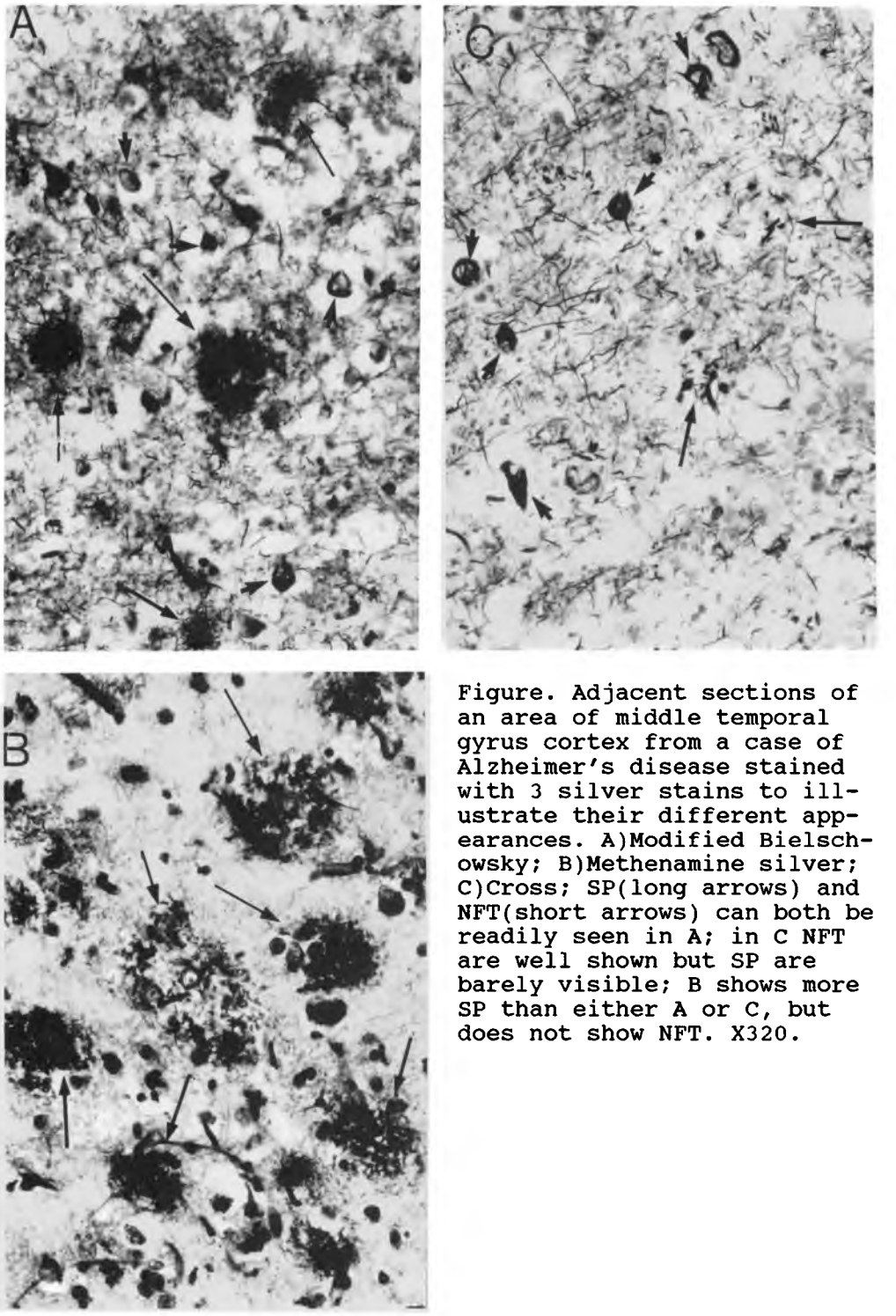



Figure. Adjacent sections of an area of middle temporal gyrus cortex from a case of Alzheimer's disease stained with 3 silver stains to illustrate their different appearances. A)Modified Bielschowsky; B)Methenamine silver; C)Cross; SP(long arrows) and NFT(short arrows) can both be readily seen in A; in C NFT are well shown but SP are barely visible; B shows more SP than either A or C, but does not show NFT. X320.

The Bielschowsky stain and, in the author's experience, the von Braunmuhl stain show both these structures well, but are not easy to perform. Some immunostaining methods have been found to demonstrate only a proportion of traditionally stained NFT and SP , at least in paraffin sections, so are not suitable for obtaining a reliable estimate of their total numbers.(Connolly et al. 1987; Duykaerts et al. 1987) On the other hand, one immunoreaction, with an antibody to beta amyloid protein, shows areas of focal cortical staining in a much larger proportion of elderly brains than is affected by conventional SP and NFT formation (Davies et al. 1988). If this reaction was used indiscriminately it might be expected to overestimate the prevalence of AD. Thus, Davies et al. (1988) found cortical foci of reactivity with this antibody in nearly 80% of autopsy brains from unselected subjects at the end of the ninth decade, whereas only 19% of the same brains showed AD pathology using conventional stains.

Another possible pitfall in the application of the Khatchachurian criteria lies in their failure to specify precisely those areas of the neocortex to be examined, stating only that frontal, temporal and parietal lobes should be examined. It has long been known that the primary motor cortex in the precentral gyrus of the frontal lobe and the somatosensory cortex in the postcentral gyrus of the parietal lobe are little affected by NFT formation and less affected by SP formation than other parts of these lobes (Pearson et al. 1985; Rogers and Morrison 1985). Likewise, in the temporal lobe the primary auditory cortex has been shown not to develop NFT in AD (Esiri et al. 1986). Therefore these areas should be excluded from "random" SP and NFT counts on frontal, parietal and temporal neocortex respectively, and it would be preferable if such counts were made on defined Brodmann areas. If the areas chosen were ones that are frequently biopsied the experience gained from such autopsy studies might profitably be applied, albeit with some caution, to the biopsy diagnosis of AD. In this context it is of interest that in 5 cases of AD that underwent both cerebral biopsy and autopsy there was no consistent change in density of SP or NFT between the time of biopsy and death (Mann et al. 1988).

One difficulty for some investigators about the Khatchachurian criteria is the suggestion that the threshold for the number of plaques required to be present for a diagnosis of AD to be made should be changed depending on whether a clinical diagnosis of dementia has or has not been made. Further uncertainty is caused by the lack of any agreement about how far the threshold should be revised downward if dementia is clinically apparent. These uncertainties, however, do no more that acknowledge the depth of our ignorance; and it must be for future studies to discover whether they can be eliminated. Another cause for concern for some is the fact that, in attempting to validate neuropathological criteria for the diagnosis of AD, attempts are made to maximise agreement with the clinical diagnosis, instead of the neuropathology itself providing the "gold standard" for the clinical diagnosis. However, it seems not

unreasonable that attempts to refine both clinical and pathological criteria should proceed hand-in-hand and take account of advances that are made in both fields. In the absence of any clear definition of what AD is, its pathological and clinical aspects need to be considered together.

## CONCLUSIONS

The difficulties in establishing reliable criteria for the biopsy or autopsy diagnosis of AD are ultimately bound up with our uncertainty about whether AD is a disease, or an inevitable consequence of ageing, with those clinically affected merely representing those more severely or earlier afflicted by the ageing process, whatever that is. Opinions on this question differ (Brody 1982; Bondareff 1982; Berg 1985; Katzman 1988; Brayne and Calloway 1988; Lancet Editorial, 1989), and the facts on which it might reasonably be settled are not available. It might help if we knew whether , in an unselected population of elderly people, the numbers of SP in the neocortex follow a normal or a bimodal distribution. If there is a normal distribution the task of establishing a valid quantitative distinction between AD and "no AD" in any individual case is likely to be harder, and perhaps less meaningful, than if there is a bimodal distribution. There are few studies that have attempted to address this question (Blessed et al. 1968; Peress et al. 1973; Millar et al. 1984) and it becomes harder to answer the lower hospital autopsy rates diminish. Meanwhile, such neuropathological criteria as we have can be used to help to validate other less invasive measures of the AD process, such as biochemical alterations in blood or cerebrospinal fluid, and radiological scanning techniques. These in turn may eventually be applied to large numbers of elderly subjects to help answer the underlying question of the inevitability or otherwise of the development of AD with advancing age.

## APPENDIX

The following guidelines are offered to those faced with the need to make a diagnosis of AD based on appearances in a cerebral biopsy (see also Esiri and Oppenheimer (1989) chapter 4):

1) A biopsy of frontal or temporal neocortex (e.g. of Brodmann areas 46 or 22) should be obtained that includes cortex and underlying white matter.

2) It should be fixed in neutral, buffered formalin and separate samples preserved for cryostat sections and electron microscopy in case these are subsequently required. As a precaution, it is wise to treat these specimens as infective as occasional cases of progressive dementia, particularly in younger patients, may be found to be due to human immunodeficiency virus infection, progressive multifocal leucoencephalopathy or Creutzfeldt-Jakob disease.

3) The formalin-fixed sample should be embedded in paraffin wax and sections cut perpendicular to the pial surface.

4) Sections can be stained with haematoxylin and eosin,

luxol fast-blue/cresyl violet (or a similar cell and myelin stain), phosphotungstic acid/haematoxylin (or a similar glial stain), a stain that has been shown to be among the best for demonstrating SP (thioflavine S, methenamine silver) and one that has been shown to be among the best for demonstrating NFT (Bodian, Cross' modification of the Palmgren) or the Bielschowsky stain, which shows both SP and NFT well in the same section (not necessarily the best way to count both structures; separate sections for each makes counting easier because there is less "noise"). In addition, the congo red stain is useful to show amyloid deposits in leptomeningeal and cortical blood vessel walls.

5) Until clearly superior criteria are produced, the quantitative criteria with regard to SP for the diagnosis of AD put forward by Khatchachurian (1985) should be applied. Particularly if no NFT are present, and SP are present only in numbers that fall short of those required to make a diagnosis of AD, the biopsy should be carefully studied for evidence of another dementing process, e.g. Lewy body dementia, some form of vascular disease, Creutzfeldt-Jakob disease, or some form of neoplastic, paraneoplastic, demyelinating, metabolic or infective process.

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# Plaques, Tangles and Amyloid:
## What Do They Tell Us about Alzheimer Disease?

Robert G. Struble
*Departments of Psychiatry and Pathology*
*S.I.U. School of Medicine, Springfield, IL*

H. Brent Clark
*Department of Laboratory Medicine, Memorial Medical Center*
*Deprtment of Pathology, S.I.U. School of Medicine, Springfield, IL*

INTRODUCTION

Currently, the critical step in the diagnosis of Alzheimer disease (AD) is neuropathologic examination of biopsy or autopsy material. The results of the neuropathologic examination determine if the lesions are consonant with a diagnosis of AD. However the relationship of these lesions to the disease process is unclear, and it is important that those performing research in AD appreciate the limitations of the neuropathologic diagnosis. Therefore, the focus of this chapter is to present an overview, for the non-neuropathologist, of the terminology used to describe lesions seen in AD, to point out deficiencies in our understanding of these lesions, and to present results of recent research into the possible etiology of these lesions.

CRITERIA FOR DIAGNOSIS OF AD

The NINCDS-ADRDA criteria for Alzheimer's disease (McKhann et al., 1984) have three diagnostic categories; probable, possible and definite. The first two categories are clinical while the final category, "definite," is a neuropathologic diagnosis based on autopsy findings of senile plaques (SP) and neurofibrillary tangles (NFT) in significant numbers. These are working criteria for the post-mortem diagnosis of AD. "Significant numbers" is a relative term, as the neuropathologic working criteria for AD are age-adjusted (Khachaturian, 1985); a greater density of SP is required for a diagnosis of AD with increasing age of the patient at the time of death. Somewhat contrary to these guidelines were quantitative studies that have shown that the density of neocortical pathology in demented individuals tended to be less with increased age of death (Jamada and Mehraein, 1968; Mann et al, 1985; Hansen et al, 1988).

The density of SP and NFT usually is high in patients diagnosed with AD; cases of demented individuals not meeting the neuropathologic criteria for AD, or where an alternative diagnosis cannot be made are uncommon (see Joachim et al, 1988). A more common problem are cases of "controls" who display borderline diagnostic pathologic changes with little or no historical evidence of dementia. In one study, these partially affected "controls" comprised up to 30% of individuals over the age of 80 living in a nursing home. These cases were found to have neuropathologic changes and neurotransmitter levels intermediate between those of demented individuals and those of non-demented controls who did not have marked cortical pathology (Katzman et al, 1988). It is the problem presented by non-demented controls with AD-like changes that led to the adoption of age-related increases in cortical pathology for the diagnosis of AD.

Over the last ten years much has been learned about the pathologic markers of AD and although SP and NFT are the accepted criteria for AD, it is unclear what disease processes underlie their formation. In addition, it is unclear whether these classical markers of AD are directly related to the dementia that characterizes AD or whether there is some other critical factor. At present, perhaps the best approach for developing a clearer understanding of possible underlying pathologic processes in AD is to describe these lesions, characterize the participating elements and attempt to understand their evolution without making premature assumptions or hypotheses about their role in the pathophysiology of dementia.

## NEUROFIBRILLARY TANGLES

Neurofibrillary tangles (NFT) are classically identified by histologic staining with silver. In addition to silver stains, NFT are also stained by thioflavin or Congo red, both of which are dyes that bind to protein in a ß-pleated sheet conformation (i.e., amyloid). The possible relationship of the NFT and amyloid deposition in the neuropil will be more fully examined in the discussion of amyloid below.

At the light microscopic level NFT are neuronal-sized collections of stained fibrils. They can assume many shapes, some of which are determined by the orientation of the neuron in the tissue section. In the neocortex NFT appear flame-shaped, anchored at the base of the neuron and extending into the apical dendrite. However, NFT can also appear globose with no clear orientation, especially when present in subcortical nuclei. Electron microscopic studies (Kidd, 1963; Terry et al, 1964) disclosed that NFT in AD are composed of both straight filaments and paired helical filaments (PHF). The PHF have a diameter of approximately 20nm, with a helical periodicity of approximately 80nm; the straight filaments have a diameter of approximately 10nm.

The terms NFT and PHF are often used interchangeably but this is not justified and leads to some confusion. In certain degenerative diseases, NFT do not necessarily contain PHF. For example, the NFT of progressive supranuclear palsy (PSP) are composed of straight filaments and only rarely are PHF seen (Tellez-Nagel and Wisniewski, 1973; Bancher et al, 1987; Tabaton et al, 1988).

Therefore, it should not be assumed that a NFT, **which is defined by its argyrophilia (i.e., silver-staining)**, is composed of PHF, **which are defined by their electron microscopic appearance.**

The relation of these clearly abnormal accumulations of fibrillary material to the dementia of AD is problematic. The Khachaturian criteria for AD include NFT in patients who die at a young age, although they are not obligatory for diagnosis of AD in patients who die at an older age. Not all cases of AD, and particularly those of late onset, display argyrophilic NFT in neocortical sections (Terry et al, 1987). Therefore, neocortical NFT are a variable feature of AD and their presence in neocortex is not required for the final diagnosis of the disease.

NFT are not restricted to AD as silver-stained, intraneuronal fibrillary structures are found in a number of diseases. A pedigree with an inherited form of dementia has been described which is characterized by NFT with no SP (Bird et al, 1989). Whether this form of dementing illness represents a variant of AD or a separate disorder is unclear. NFT are assumed to underlie clinical manifestations of several diseases such as ALS-Parkinson-dementia complex of Guam (Hirano et al, 1961), dementia pugilistica (Corsellis et al, 1973), and PSP (Tellez-Nagel and Wisniewski, 1973). They also have been found in, but are not diagnostic of, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis (Wisniewski et al, 1979) and sudanophilic leukodystrophy (Harada et al, 1988). NFT have also been identified in aged bears and monkeys (Cork et al, 1988; Cork et al, 1989). The presence of NFT in the nucleus basalis of Meynert, ipsilateral to a massive cerebral cortical infarct (Kato et al, 1988) suggests that they may develop subsequent to loss of target neurons since the nbM projects ipsilaterally to cerebral cortex (Jones, 1983).

There is no question that the presence of NFT represents evidence of neuronal dysfunction. Electron microscopic studies (Sumpter, 1986) suggest that NFT occupy significant amounts of the perikarya and that the nucleus shrinks. This perikaryal engorgement almost certainly compromises neuronal function. Compromise of neuronal function, short of actual neuronal death, could result in some of the cognitive abnormalities seen in AD. Therefore, a major interest in research has been to determine which subcellular elements comprise the NFT.

Numerous studies have linked the NFT to abnormalities of cytoskeletal constituents. Immunohistochemical staining for epitopes of phosphorylated and non-phosphorylated neurofilaments, and two microtubule-associated proteins, MAPII and tau ($\tau$), has been observed in NFT (Anderton et al, 1982; Dahl et al, 1982; Cork et al, 1986. Grundke-Iqbal, et al, 1986; Joachim et al, 1987 Ksiezak-Reding 1987). The PHF may display antigenicities that are unique (Ihara et al, 1983) and in addition, contain epitopes of multiple cytoskeletal related proteins (Ihara et al, 1986; Grundke-iqbal, et al, 1986; Kosik et al, 1986; Mulvihill and Perry 1989). Wischik et al, (1988) reported that a monoclonal antibody directed against $\tau$ stained PHF but only after digestion of isolated PHF with pronase. That observation suggested that the antigenic site was not exposed in the native conformation of the PHF. They also noted

that there is a much larger residual portion, as viewed by EM, of the PHF still uncharacterized.

The precise antigenic composition of a NFT may be a function of the region of the brain in which they are found. Subcortical NFT in both AD (which have PHF) and PSP (which do not typically have PHF) were positively stained with a battery of cytoskeletal antisera, including an anti-"PHF" antiserum. Anti-$\tau$ and anti-neurofilament antisera stained a greater percentage of cortical than subcortical NFT in both AD and PSP, suggesting that the location or the connectivity of neurons may influence their antigenic composition (Tabaton et al, 1988). However, in another study, anti-"PHF" antisera did not stain the NFT of PSP (Bancher et al, 1987).

Negative results with immunohistochemistry should always be interpreted conservatively since failure to identify an epitope does not mean it is not present. As Wischik et al, (1988) showed, pre-treatment of PHF with pronase modified immunoreactivity by unmasking $\tau$-like immunoreactive sites. Positive staining similarly should be interpreted carefully; an epitope common to several proteins could complicate interpretations of immunoreactivity. For example, an antibody raised against neurofilaments also has been shown to recognize $\tau$, presumably because both proteins have comparable amino acid domains or perhaps slight cross-contamination of the neurofilaments with $\tau$ (see Ksiezak-Reding et al, 1987). In sum, there is ample evidence of involvement of cytoskeletal elements in both NFT and PHF. However, the interpretation of studies providing that evidence must be tempered by the realization of the methodologic limitations inherent in immunohistochemistry.

Recent interest has been focussed on A68, an abnormal protein abundant in AD (Wolozin et al, 1986). A68 has a molecular weight of 68kD and is recognized by a monoclonal antibody, Alz-50. A68 has been suggested to be an early marker of NFT (Hyman et al, 1988a) since, in addition to staining NFT-shaped structures, Alz-50 antibody also labelled perikarya that did not have a clearly formed NFT. Further, many more positively-stained neurons were seen than with standard NFT stains such as thioflavin or silver. However, Alz-50 immunoreactivity is transiently enriched in neonatal neurons, a population in which at least some neurons will go on to die presumably without forming a NFT (Wolozin et al, 1988; Hamre et al, 1989). Recent reports suggest A68 may be a species of $\tau$ (Ksiezak-Reding et al, 1987, 1988; Nukina et al, 1988) and that presence of Alz-50 immunoreactivity is associated with decreased protein synthesis (Doebler et al, 1988). In sum, the significance of A68 in a neuron is unclear. It presence in neurons could be an early marker for formation of the NFT or may signify a dysfunctional neuron.

It also is not proven that abnormal perikaryal accumulations of cytoskeletal proteins detected by immunocytochemistry necessarily evolve to the NFT. Inspection of sections from AD brain stained with anti-cytoskeletal antibodies, including anti-phosphorylated neurofilament antibodies, suggest that not all of the cytoskeletal antigens detected are associated with NFT; many individual neurons display an amorphous accumulation of antigen. Experimental studies in rats have shown that within days of a

peripheral axotomy excess phosphorylated neurofilament epitope appeared in the dorsal root ganglion neurons and staining rapidly disappeared as the axon began regenerating (Rosenfeld et al, 1988). This study suggested that the perikaryal presence of phosphorylated neurofilament epitope in rats was secondary to a peripheral axotomy. The abnormal accumulation of phosphorylated neurofilament epitope (and perhaps other cytoskeletal markers) in AD may represent an early sign of NFT formation or may represent relatively non-specific neuronal disruption. It is possible that the excess of cytoskeletal epitopes found in AD perikarya can either evolve to a NFT (PHF?) or conversely, the neuron may die without forming a NFT. However, no definitive evidence exists bearing on this question.

Finally, the fate of the NFT is unknown. The failure to observe neocortical NFT in all cases of AD, and particularly those cases with a later age of onset (Terry et al, 1987) suggests at least three hypotheses: a) that different etiologies or manifestations of AD may exist, some of which do not result in neocortical NFT formation; b) that NFT are formed over a prolonged period of time and the shortened survival in an aged onset population does not permit formation; or c) that NFT may actually disappear or else become cryptic to standard stains. Specifically, it is unclear whether NFT will disappear during the course of the disease. "Ghost tangles" (i.e., with no signs of a perikarya) can be difficult to detect and it is possible that NFT may be processed to an invisible form. The association of glial fibrillary associated protein immunoreactivity with extracellular NFT (Schmidt et al, 1988) suggests that glia might degrade the NFT. Conversely, the insoluble nature of PHF (see Selkoe, 1986) suggests they would remain in the neuropil although special stains might be necessary for detection.

The question of NFT evolution is further highlighted by comparison of biopsy-obtained samples with autopsy-obtained tissue from the same patients (Mann et al, 1988). This work demonstrated no systematic increase in the density of NFT from biopsy to autopsy (Mann et al, 1988). These observations can be interpreted to suggest that NFT cease to form between the biopsy and autopsy (perhaps related to local trauma at the site of the autopsy) or that early-formed NFT may disappear during the course of the disease and are replaced by a new NFT. It also possible that the small biopsy sample (<0.5cc) was inadequate for a representative sample of pathology. It would be worthwhile determining the variability of lesion density to be expected in multiple samples from within the same cortical region.

In sum, although NFT are one of the criteria for the diagnosis of AD, relatively little is known about their pathogenesis, fate over the course of the disease, or functional consequence to the central nervous system. While numerous cytoskeletal epitopes have been identified on NFT and PHF, their identification may reveal little about the causative agent. It is worth re-emphasizing that NFT are found in several storage diseases (Wisniewski et al, 1979; Harada et al, 1988) making their interpretation problematic. Multiple etiologies (e.g., axotomy by amyloid accumulation or post-synaptic neuronal degeneration) could result in a common cellular response. Perhaps the NFT in AD and other degenerative diseases

**reflects** neuronal dysfunction which leads to cytoskeletal abnormalities. The NFT may be an epiphenomenon of some other degenerative process. These questions need to be addressed in subsequent studies of AD and other dementing illnesses.

GRANULOVACUOLAR DEGENERATION AND HIRANO BODIES

While these two elements are not required for the diagnosis of AD, it is generally appreciated that they are present in increased numbers in AD (Ball and Lo, 1977; Mann, 1978). Granulovacuolar degeneration (GVD) is characterized by an eosinophilic core surrounded by a cleared vacuolar area within the cytoplasm of the neuron. While most common in the hippocampal region, GVD is occasionally present in other cortical regions. In addition, some neocortical neurons contain small eosinophilic spots that may represent the cores without the clear vacuole (Tomlinson and Kitchener, 1973). Immunohistochemical staining of GVD has been obtained with antisera to tubulin, neurofilaments and $\tau$ (Price et al. GVD; Dickson et al, 1987), suggesting that, like NFT, disorders of the cytoskeleton are involved with GVD formation.

Hirano bodies (HB) (Hirano et al, 1968) are another histologic abnormality that have an increased frequency in AD (Gibson and Tomlinson, 1977). These eosinophilic structures, typically found in dendrites of hippocampal neurons, have been labelled with antisera to the cytoskeletal protein, actin (Goldman, 1983; Galloway et al, 1987) and neurofilament-like immunoreactivity of HB has been reported (Schmidt et al, 1989).

In sum, NFT, GVD and HB all represent apparent disorganization of the cytoskeleton. However, documenting the presence of cytoskeletal abnormalities does not necessarily imply that cytoskeletal dysfunction is the cause of AD. Rather, abnormalities of cytoskeletal proteins could be a secondary response to some unknown primary cause.

SENILE PLAQUES

The term "plaque", used in neuropathology, (with the exception of multiple sclerosis plaques) usually refers to a circular (spherical) defect in the neuropil with a diameter in the range of 20-120 $\mu$m. However, the term "plaque" is generic referring to neuropil lesions in multiple types of dementing illnesses. The "Kuru-like" cortical plaques of Creutzfeldt-Jakob disease (caused by a transmissible agent) are usually neurite-free; they do contain "amyloid" by conventional stains although this "amyloid" is derived from a different precursor than that found in AD (Kitamoto et al. 1986; Masters et al, 1985; DeArmond et al, 1987). Recently a family with putative familial AD and "plaques" has been reported to actually have a transmissible encephalopathy like Creutzfeldt-Jakob disease (Nochlin et al, 1989) and a recent report suggests the existence of an inherited dementing disorder that forms plaques composed of neither A4 peptide (see below) nor the peptide characteristic of Creutzfeldt-Jakob disease (Rosenberg et al, 1989). Hence, one should be circumspect in the usage and interpretation of the term "plaque".

The term senile plaque (SP) usually is restricted to AD plaques. SP are the most consistent pathology used to diagnose AD and much work has focused on understanding their pathogenesis. SP are composed of two principal elements, neurites and amyloid, (Kidd, 1963; Terry et al, 1964), each of which is discussed below. In addition to these elements, glia are often encountered in SP, which is not surprising as the SP is a focal site of degenerating structures and a glial response would be expected. Synonymous terms (although not always) are neuritic plaques, argyrophilic plaques or amyloid plaques. We will confine ourselves to the generic term senile plaque (SP), as the other terms have been used in discussing both the appearance and pathogenesis of SP.

Neurites: Neurite is a general term for axonal and dendritic extensions from the perikarya and does not necessarily signify an abnormal process. However, when used in the context of AD, the term 'neurites' usually refers to abnormally large or misshapen structures in abnormal distributions. These neurites are usually detected using silver-stains, appearing as swollen structures up to 20$\mu$m in diameter. Because some routinely used silver-stains detect neurofilaments (Gambetti et al, 1981), presumably many neurites represent dystrophic axons (Wisniewski and Terry, 1973), some of which are axonal terminals. Combined silver-staining and immunohistochemical techniques (Walker et al, 1988), and electron microscopic studies (Wisniewski and Terry, 1973; Struble and Cork, personal observations) detect many more swollen, dystrophic fibers in a SP than can be seen with a silver stain alone. This finding is not surprising as several studies report dendritic processes in SP (Scheibel and Tomyasu, 1978; Probst et al, 1983) and dendritic components may remain unstained by most routinely used silver stains.

As is the case with NFT, abnormalities of the cytoskeleton are implicated in SP-associated neurite formation (see Selkoe, 1986). It seems probable that SP neurites, at least in part, are supplied by neurons containing cytoskeletal abnormalities. Probst et al, (1989) reported that in regions of AD cortex that lack NFT, SP neurites also lacked anti-PHF and $\tau$-like immunoreactivity. This observation suggests that the composition of the SP may vary as a function of local neuronal pathology and that multiple local factors might determine certain characteristics of SP.

One approach to investigating the etiology of the SP was identifying the neurotransmitters in SP-associated neurites. These studies were based on the assumption that knowledge of neurotransmitter markers in neurites would predict which neurotransmitter systems were at risk in AD and perhaps elucidate mechanisms by which transmitter-specific systems degenerated in AD. Initial histochemical and immunocytochemical studies on aged monkeys detected acetylcholinesterase, choline acetyltransferase and somatostatin associated with SP neurites (Kitt et al,1984; Struble et al, 1982; 1984a,b). Evidence for somatostatin also was found in human SP (Armstrong et al, 1985; Morrison et al, 1985; Kowall and Beal 1988). Over the last several years neurites containing tyrosine hydroxylase, dopamine-$\beta$-hydroxylase, glutamic acid decarboxylase, corticotropin releasing factor, leucine enkephalin, cholecystokinin, neurotensin, vasoactive intestinal peptide, substance P, and neuropeptide Y have been reported to be associated

with SP (Chan-Palay et al, 1985; Kitt et al, 1985; Walker et al, 1985; Powers et al, 1987; Struble et al, 1987; Powers et al, 1988; Kowall and Beal, 1988). Localization of two (or more) neurotransmitters in separate neurites in the same SP has been shown (Walker et al, 1988); neurites containing either NPY or TH were found in the same senile plaque.

In sum, it appears that many, if not all, neurotransmitter-specific systems, including those not previously known to be at risk in AD (e.g. cholecystokinin and substance P: Crystal and Davies, 1982; Ferrier et al, 1983) can contribute neurites to SP. The transmitter markers observed in plaque-associated neurites appear to be related primarily to the neurotransmitter systems present in the region where the SP are found, rather than to any propensity for particular transmitter systems to form plaque-associated neurites. Perhaps the best examples of this phenomenon are for DBH (Powers et al, 1988) and neurotensin (Struble et al, 1987). Heavy DBH-like immunoreactivity is found in the CA4 region of normal hippocampus and is present in most SP in this region; neurotensin immunoreactivity is found in the subicular region of normal hippocampus and is present in neurites of SP in this region.

The above observations strongly suggest a non-specific origin of neurites in SP and support the suggestions that some of the classical neuritic changes in AD may represent reactive sprouting (Geddes et al, 1985; Hyman et al, 1987; Struble et al, 1987). Early studies (Ramon y Cajal, 1923) described sprouting in experimentally transected axons which resulted in filopodia that resemble SP neurites. In silver stains of tissue from a case of AD, one of us (RGS) identified an axon displaying filopodia along its length, and was able to trace the axon to a SP. The concept of reactive sprouting should raise questions about possible therapies that would induce or support sprouting because over-stimulation of sprouting could paradoxically increase the progression of one of the classic manifestations of the disease.

Amyloid: The second major element in the SP is amyloid. This material is classically visualized with dyes such as congo red or thioflavin which have a high affinity for proteins with a $\beta$-pleated sheet configuration. Over the past several years our understanding of the diversity and origins of amyloid has greatly improved, although numerous questions still exist. The term "amyloid" is generic, i.e., it refers to any proteinaceous material deposited in a $\beta$-pleated sheet, that stains with dyes that have binding affinity for that type of proteinaceous secondary structure. The primary source of the proteins forming the amyloid is not disclosed by the conventional amyloid stains and can include immunoglobulins, prealbumin and a number of other proteins (see Hind, 1986).

Of specific interest for understanding AD is $\beta$-amyloid, a brain-specific protein that accounts for the majority of amyloid seen in the CNS (Glenner, 1980; Eikelenboom and Stam, 1984; Hind, 1986). Deposits of $\beta$-amyloid, composed of the A4 protein, are found in cerebral vascular amyloidosis (CAA) and in SP of both AD and Down syndrome (Glenner and Wong, 1984; Masters et al, 1985). The A4 protein is a small, 4kD, protein that apparently is degraded

from larger precursors with molecular weights ≈695-750kD (Tanzi et al, 1988; Ponte et al, 1988; Kitaguchi et al, 1988). These larger species, the amyloid precursor proteins (APP), represent alternatively spliced forms transcribed from the APP gene. The 750kD form contains a Kunitz-like serine protease inhibitor while the 695kD form does not have this sequence. The deposited ß-amyloid is suggested to be formed from the transmembrane domain (A4 protein) of the larger precursor, with the intra- and extracellular elements cleaved during degradation (Kang, 1987; Dyrks 1988).

The relationship between β-amyloid and the NFT is the subject of much debate. Immunohistochemical studies suggest that extracellular amyloid may be similar to proteins found in NFT and PHF (Masters et al, 1985; Kidd et al, 1985), although either low-level contamination of NFT/PHF fractions by β-amyloid or amino acid sequence homology may lead to cross-reactivity of antisera (see Selkoe 1986). Defossez and Delacourte (1987) present data supporting transition of NFT to β-amyloid and Cole et al, (1989) stained neurons and SP neurites in AD with an antisera raised against the A4 amino acid sequence. APP is reported to be present in axons, dendrites, perikarya and neurites of some SP in aged rhesus monkeys (Martin et al, 1989). At present, the association among β-amyloid, PHF and NFT is unclear although the preliminary results noted above are suggestive of some type of relation.

One major impetus for focusing research on the A4 protein was the finding that the gene for APP mapped to chromosome 21 (Tanzi et al, 1987; Goldgaber et al. 1987) as did the AD linkage marker for four pedigrees of familial AD (FAD) (St. George-Hyslop et al, 1987; although see Schellenberg et al, 1988). Subsequent studies disclosed that the APP gene is separate from that of the FAD gene (Van Broeckhoven et al, 1987) arguing for the APP gene not being the cause of familial AD.

The localization of β-amyloid both in the neuropil and in blood vessels, raises further controversy about the origin of the A4 peptide. Some suggest that it originates from blood vessels and spreads to the brain parenchyma, while others suggest that its presence in blood vessels is secondary to initial accumulation in the parenchyma. This dichotomy will be discussed further below.

In addition to the A4 peptide isolated from plaque cores, numerous other markers have been found including IgG, IgM, albumin and several globulins (Powers et al, 1981). Heparin sulfate proteoglycans have been immunohistochemically identified on the β-amyloid fibrils both in plaques and in cerebral amyloid angiopathy suggesting involvement of the extracellular matrix (Snow et al, 1988). Schubert et al, (1988), on the basis of amino acid sequence analysis, suggested that the β-protein precursor might be a heparin sulfate proteoglycan core protein. The presence of these other peptides in β-amyloid could result from either participation in SP formation, or entrapment of local peptides in the highly insoluble β-amyloid.

Recent research has detected $\alpha_1$ anti-chymotrypsin in SP amyloid deposits (Abraham et al, 1988). This protease inhibitor might block β-amyloid degradation and lead to the formation of

these extracellular deposits. Finally, there is evidence, although somewhat conflicting, that alternatively-spliced mRNA for APP might lead to abnormal amounts of a Kunitz-type protease inhibitor in cases of AD (Johnson et al, 1988; Palmert et al, 1988). The abnormal presence or distribution of such protease inhibitors may lead to abnormal processing of the APP (see Cole et al, 1989) and to tissue deposition of β-amyloid; but directly relating this to neuronal death is speculative at the moment.

The precursor of β-amyloid is not a 'brain-unique' protein. Immunocytochemical evidence for the protein has been found in muscle cells (Zimmerman et al, 1988) and mRNA for the A4 protein or APP precursor has been found in numerous non-neural tissues such as thymus (Tanzi et al, 1987). Finally, immunohistochemical studies suggest that the β-amyloid protein is conserved among numerous species (Selkoe et al, 1987).

The normal function of APP is unknown, although it may function as a cell surface receptor (Kang et al, 1987). Recently, a polypeptide identical to the 1-28 amino-terminal fragment of the A4 protein amino-acid sequence has been reported to improve survival of neurons in tissue culture suggesting a trophic role for this polypeptide (Whitson et al, 1989). In contrast, the carboxy-terminus domain of APP, when over expressed in a transgenic PC12 culture, resulted in toxin formation that caused death of cultured hippocampal neurons. This toxicity could be absorbed by an antiserum to APP (Yanker et al, 1989). The significance of these early reports is unclear but does suggest that abnormal abundance of transcripts of A4 or APP can modify survival of neurons in culture.

Even if β-amyloid proves not to be the critical factor in the pathogenesis of AD, it presents an interesting element to study for its own sake. It seems probable that the amyloid precursor protein functions in some important, normal cell-cell interaction, although how is presently unclear. The processes that lead to A4 deposition with subsequent β-amyloid formation in the neuropil in AD probably are of important consequence to the development of AD and may underlie some of the clinical symptomatology. However, we must also be cautious of over-interpreting the importance of β-amyloid in the pathology of AD as this is a relatively new field of study.

Evolution of the Senile Plaque: The evolution of the SP is of interest for several reasons. Proper staging of the various forms of the SP may provide information on the natural course of the disease. Staging could also provide information on duration of involvement of a cortical region, perhaps leading to a better appreciation of the relationship of SP to clinical symptoms. To this end, several papers have examined the possible evolution of the SP.

An initial stage of AD-type cortical SP formation has been suggested to be represented by circular disruption of the neuropil, without evidence of β-amyloid or abnormal neurites (Probst et al, 1987). Stereologic estimates also suggested that each of these "early" SP had a microglia at its core. The distribution and amorphous nature of this type of plaque led those authors to suggest that it might presage the formation of a classic SP

containing both neurites and β-amyloid. The predominance of "early" SP, in the absence of the classic SP, in specific cortical areas was explained by proposing that the evolution of the SP was arrested. If this neuropil abnormality does indeed represent a primitive plaque, and that continuation to a classic SP can be arrested, it suggests that SP formation is regulated by local factors, and that the type of SP formed (or whether they are formed at all) is largely determined by the cortical neuropil. This hypothesis is comparable to that suggested for the transmitter-specificity of neurites in plaques, i.e., it is largely related to the local characteristics of the neuropil. Finally, antisera raised against the A4 protein have detected clusters of immuno-staining not visualized with thioflavin, Congo red or routine silver stains. These reports suggest that an early stage of senile plaque formation (Tagliavini et al, 1988; Braak et al, 1989) might be characterized by deposits of β-amyloidogenic peptide not yet in a β-pleated sheet.

There probably is an evolution of SP from one form to another, but few strong data exist for concluding that "primitive" plaques evolve to "classic" plaques leading to "cored" plaques. What is known is that there are variable amounts of amyloid associated with neuritic abnormalities. Wisniewski and Terry (1973) first suggested that SP displayed a progressive evolution from a form characterized by swollen dystrophic neurites with little or no detectable amyloid (primitive plaque), maturing to a SP containing both neurites and a core of amyloid (classic plaques), and reaching senescence as a cored, amyloid-rich senile plaque with few or no neurites (end-stage or burned-out SP). This evolution was partially supported by quantitative studies of aged Rhesus monkeys (Struble et al, 1985) which showed that as the density of SP increased, the amount of amyloid in each senile plaque also increased. A combined biopsy-autopsy study in patients with AD did not find evidence that SP density or type markedly changed over the course of several years (Mann et al, 1988) although there was a slight, but not consistent, increase in the amount of amyloid present in SP from biopsy to autopsy.

SP with prominent cores were rarely encountered in Rhesus monkeys studied by Struble et al, (1985); this observation was supported in subsequent observations (Struble, personal observations). Conversely, recent work (Lary C. Walker, personal communication) demonstrated the predominant type of SP in squirrel monkeys was characterized by an amyloid core with a paucity of argyrophilic neurites. In contrast to the Rhesus monkeys (in which cerebral amyloid angiopathy was rare) CAA was prominent in these squirrel monkeys. This species difference in presence of CAA and type of SP suggests that there may be an interaction between CAA and SP core formation, although this proposal is speculative.
Further studies of AD have suggested that SP with the "classic" circular (spherical) core of amyloid are rather rare. In a study of three pedigrees of familial AD (Struble et al, 1987), β-amyloid cored plaques in hippocampus were prominent in only two of three pedigrees; when present, they were almost exclusively found in CA4 although highest densities of SP were found in CA1-2. Cored plaques comprised only 6% of the SP in the hippocampus. In a subsequent study of hippocampus from thirty cases of AD (Struble et al, 1988; Becker et al, in prep), three quarters of the cases

examined had some SP with a marked core, but the density of these SP was low and generally restricted to CA4. These observations suggest that the cored senile plaque is not an aberrant manifestation of AD, but that it does make up only a small percentage of total SP.

In sum, the progression of a SP from neurite-rich form to an amyloid-rich form, or conversely, cannot be conclusively determined. This is especially true if there are stages of SP cryptic to routine stains. While knowing the evolutionary pattern of the SP would be valuable, too few data exist for determination of progression. In addition, the assumption of the unitary nature of all SP in AD may not be justified. Much as NFT, SP formation may represent a response in the neuropil to some insult and not necessary represent the primary abnormality of AD.

CEREBRAL AMYLOID ANGIOPATHY

As noted earlier, a major question is the source of β-amyloid; i.e., whether it is derived from a vascular source and released into the neuropil, or whether it is formed in the neuropil and collects in blood vessels in the CNS. Cerebral amyloid angiopathy (CAA) is characterized by vascular deposition of amyloid and for purposes of this discussion will be limited to the β-amyloid (A4 protein) of AD. We will only briefly discuss the most salient points; CAA and its relationship to AD has been excellently reviewed by Vinters (1987). The basic problem with understanding the relationship between CAA and β-amyloid deposition in AD is that reports suggest not all cases of AD display significant amounts of CAA (Gilbert and Vinters 1983) and cases of CAA exist (apart from the familial hereditary amyloidoses) where there is no evidence of SP formation (Vinters and Gilbert 1983). In addition, in some cases of AD there is no detectable deposition of amyloid in parenchymal vessels although it is seen in the pial vessels. Finally, the density of amyloid deposition and the density of AD pathology often disagree. For example, hippocampus usually displays marked pathology in AD, but it is not a classic site of vascular amyloid deposition (Morimatsu et al, 1975; Vinters and Gilbert, 1983). In sum, while there is ample evidence to suspect that CAA and the pathology of AD are closely related, it is unclear what that relationship is. One intriguing suggestion is that amyloid deposition might mark a local site of inflammatory response (Vinters, 1987). However, that author notes that it is not possible to determine if the deposition of amyloid was the result of an inflammatory response, or if an inflammatory response was mounted to the presence of the amyloid.

**NEURONAL LOSS**

Neuronal loss in AD tends to be the scientific step-sister to the more obvious pathology of SP and NFT. This probably reflects the difficulties of quantifying shrinkage and neuronal loss; relatively few researchers have been willing to undertake this difficult task. However, the literature cited below suggests neuronal loss may be an important variable underlying cognitive dysfunction.

Several studies have examined neuronal loss as a consequence of aging and AD (see Kemper, 1984). The most salient for this review are the studies of Terry et al, (1981) and Mountjoy et al, (1983). A consistent finding in the former study was in frontal and temporal cortex where there was a 40-46% loss of Nissl-stained neurons falling into the largest size category. Part of this loss could be explained by perikaryal shrinkage, as the average size of neurons significantly decreased in the AD group. Mountjoy et al, (1983) described similar findings extending observations to other neocortical areas. Taken as a whole, these studies suggested that neuronal shrinkage and/or loss was a consistent feature of AD.

NFT as the cause of the neuronal loss was addressed by Mountjoy et al, (1983) who reported a correlation ($r \approx -0.45$) between NFT densities and neuronal density. A negative correlation would be expected if NFT-bearing neurons degenerate; i.e., as more tangles developed, fewer neurons would be visible with Nissl stains. However, a correlation coefficient of 0.45 statistically "explains" only about 20% of the cell count variation between controls and cases of AD. Mann et al, (1985), using a restricted sample of only lamina III neurons, those likely to form NFT, obtained a better correlation coefficient ($r \approx -.68$) which would statistically "explain" about 46% of the variance but even in this case, much of the neuronal loss would not be easily explained by NFT formation.

It seems reasonable that neuronal shrinkage/loss could occur without tangle formation. Retrograde degeneration is a possible mechanism for neuronal loss and is supported by observations of adrenergic neuronal loss in the brainstem (Burke et al, 1988). Those authors suggested that degeneration of the locus coeruleus neurons deprived those adrenergic neurons which projected to the locus coeruleus of their terminal field, and in turn, those neurons degenerated. Hence, it is possible that neurons could degenerate either as a consequence of forming a NFT or as a result of retrograde degeneration.

If retrograde degeneration is a generalized phenomenon in AD, then this process must be considered as an anatomical basis of dementia in AD. For example, entorhinal cortex and cortical amygdala undergo severe neuronal loss in AD (Herzog and Kemper, 1980; Hyman et al, 1988b; Price et al, 1988). Other areas, such as the olfactory bulb or multimodal cortical areas that project to those areas may degenerate secondarily from loss of post-synaptic targets. Similarly the loss of neurons that is seen in the basal forebrain cholinergic system, locus coeruleus or raphe in AD (Whitehouse et al, 1981; Curcio and Kemper, 1984; Bondareff 1982) may secondarily reflect cortical degenerative changes. Reports that cortical neuronal loss correlated with neuronal loss in the nucleus basalis of Meynert and locus coeruleus (Mann et al, 1985) reinforced this hypothesis. Focus on neuronal loss (and shrinkage) is further emphasized by biopsy studies (Bowen and Neary, 1985; Mann et al, 1988). Those studies strongly suggested that neuronal loss and shrinkage were a consistent change observed early in the disease; SP and NFT were more variably expressed. In a combined biopsy-autopsy study (Mann et al, 1988) neuronal loss was the only variable that clearly progressed between biopsy and autopsy of the same cases.

REGIONAL PATHOLOGY

The hallmark of AD is cerebral cortical pathology (for review see Kemper, 1984). The primitive cortices of the hippocampus, amygdala and entorhinal regions show the greatest severity of pathology. Other neocortical areas are less extensively involved, with a general tendency for inferotemporal regions of cortex to display more severe pathology than do other neocortical regions. However, after these regions, there is discrepancy among reports of the distribution of pathology. Cortical weight loss is reported to be severe in parietal cortex (Najlerahim and Bowen, 1988); Brun and Englund (1981) report severe pathologic changes, including gliosis, spongiosis and "laminar blurring" (in addition to SP and NFT) in the parietal lobe. Severe involvement of parietal cortex is further supported by some clinical manifestations of AD such as apraxia and aphasia. Positron emission tomography tends to support this localization with earlier stages of AD showing tomographic abnormalities predominantly in temporal and parietal cortex (see Parks et al, 1989).

However, these observations of parietal involvement are not fully compatible with quantitative observations of NFT and SP which suggest relatively less severe pathology in parietal cortex than in other cortical regions (Jamada and Mehraein, 1968; Terry et al, 1981; Mountjoy et al, 1983). The discrepancy between SP and NFT density and parietal involvement is resolved when reports are carefully examined; i.e., the definition of 'pathology' can determine the distribution observed. Brun and Englund (1981) used multiple measures, including gliosis and spongiosis in their rating, going beyond numerical estimates of SP and NFT density and Najlerahim and Bowen (1988) may have similarly measured this more diffuse pathology in directly weighing grey matter from controls and AD cases. However, these results do raise the question of the role of SP and NFT in deficits reported in PET scanning and neuropsychological testing.

Subcortical pathology in AD can be more variable. Subcortical SP are observed in only some cases of AD (McDuff and Sumi, 1985) and are reported in the basal ganglia in some cases (Rudelli et al, 1984). Subcortical neuronal loss and NFT in the nucleus basalis of Meynert, raphe, locus coeruleus and brainstem cholinergic neurons have been reported by numerous authors (Whitehouse et al, 1982; Mufson et al, 1988; see German et al, 1987 for review) and hypothalamic tangle formation has been reported (German et al. 1987). In sum, the subcortical changes in AD are widespread and tangles are reported to form in some nuclei that project to cortex (German et al, 1987), but at present, no consistent hypothesis explains selective vulnerability of subcortical neurons. While these subcortical changes undoubtedly contribute to the dementia that characterizes AD, it is unclear how important the involvement of these regions is to the development of the dementia of AD.

CLINICO-PATHOLOGIC CORRELATION

One underlying assumption in the study of the neuropathology of AD is that by understanding the distribution of pathologic changes, one will obtain an explanation for the dementia. However,

close examination of available data suggest that this correlation may not be particularly strong.

One study suggested that as the density of cortical SP increased, cognition declined (Blessed et al, 1968). They reported correlation coefficients of 0.77 between plaque counts and clinical dementia scores and 0.59 between plaque density and results of neuropsychological testing. However, much of this correlation was a "two-point" phenomenon; i.e., when the cognitively intact group, with few or no plaques, was removed from the analysis, the correlations dropped to 0.40 and 0.26 respectively, the latter not statistically significant. Further, a correlation of 0.40 only statistically "explained" 16% of the total variance. Wilcock and Esiri (1982) reported both SP and NFT densities and their associations with a four-point dementia scale. They found relatively little association between SP (r values ranged from 0.15-0.43) and dementia; NFTs correlated more consistently (r values ranged from 0.19-0.59) suggesting that the density of NFT better correlated with the severity of dementia.

Most interesting for interpretation of the progression of AD and the relationship between clinical symptoms and neuropathologic change are studies of brain biopsy samples from demented patients. Biopsy studies are especially relevant as they provide a close temporal linking of cognitive testing and neuropathologic exam, often not available in the end-stage AD case. In a study that included neurochemical, neuropathological and neuropsychological testing several inter-relationships were found among multiple variables (Neary et al, 1986a,b). The strongest correlations were found among psychological test results and measures of neuronal loss and indirect histologic measures of protein synthesis (nucleolar size); at best, these factors accounted for about 50% of the inter-individual variability. Density of SP and neurofibrillary tangles correlated less well with cognitive decline. These data suggest that neuronal loss and dysfunction may be better predictors of cognitive decline than are SP and NFT. Moreover, a combined biopsy-autopsy study (Mann et al, 1988) suggested that the density of SP and NFT are not directly related to the duration of the disease and studies that attempt to relate the density of these lesions to the severity of cognitive decline may be difficult to interpret.

Further complicating the interpretation of the relationship among NFT, SP and the severity of dementia are observations of patients with Down syndrome and other types of mental retardation. Individuals with Down syndrome universally develop the classic pathology of AD by middle age in densities that are diagnostic of AD (Ropper and Williams, 1980; Mann et al, 1985; Wisniewski et al, 1986). However, where information on cognitive functioning was available, only about one-third of the individuals evidenced decreased cognitive function that could be interpreted as dementia. Reanalysis of data presented by one study (Wisneiwski et al, 1986) suggests that those who did develop dementia had markedly higher concentrations of SP, (almost three times more in the 61-70 y/o age group) than the age-matched, non-demented cohort. This trend was not obvious for NFT.

Studies of mental retardation, not associated with trisomy 21, have found densities of SP and NFT that are indicative of AD-type dementia in a greater proportion than would be expected in the general non-retarded population (Barcikowska et al, 1989). However, there was no clear quantitative association of AD-type pathology with evidence of cognitive decline.

In sum, these observations suggest that a threshold for developing clinically evident dementia in retarded patients might require higher densities of SP and NFT than that required in the normal population. Perhaps because of reduced levels of initial functioning, a greater degree of deterioration of the cognitive skills is required for clinical detection. Nonetheless, a simple linear relationship among SP, NFT and cognitive decline is generally not supported in AD or in individuals with AD-like changes.

## CONCLUSIONS

The classic neuropathologic lesions of AD, i.e., SP and NFT, although used to neuropathologically define the disease, display a disturbing degree of non-specificity. NFT in particular, are found in a number of dementing diseases and disease states. The specificity of SP to AD may represent a circular argument; significant numbers of SP are required for the diagnosis of AD, but without SP it is not AD but some other form of dementia. The A4 protein and cerebral amyloid represent a most interesting research subject, but the presence of CAA in patients with no AD changes, either clinically or neuropathologically, tends to argue against it being a primary cause of AD.

Further complicating the interpretation of NFT and SP is that, by and large, these classic lesions of AD do not correlate well with the severity of dementia in either autopsy or biopsy series. This is not to suggest that they are unimportant in the development of the dementia, but the relatively small amount of variation in clinical symptoms they can "account for" suggests that they may not be the critical element. The best neuropathologic predictors for the severity of dementia appear to be neuronal death and possible measures of protein synthesis dysfunction (nucleolar shrinkage). While these measures may, in turn, correlate significantly with NFT and SP, the concordance among NFT, SP and neuronal death and dysfunction appears modest. At present, the relationship of classic neuropathologic change of AD to the clinical dementia that characterizes AD is unclear.

While it is easy to survey the literature and highlight discrepancies, alternative hypotheses for the etiology of AD are more difficult to develop. Perhaps we are at a similar stage of understanding AD as we were in understanding neuronal storage diseases prior to biochemical demonstration that defects of numerous enzymes could result in similar neuropathologic presentations. The neuropathologic presentation of AD could represent several etiologic agents that result in the characteristic lesions of AD. Multiple etiologies are suggested by estimates of the heritability of AD; estimates of the percent of inherited AD range from 10-70% of AD cases (for review see Davies, 1986). The converse of these estimates is that from 30-90% of AD is not

inherited, leading to the possibility of a multifactorial causation of AD-like changes. It is possible that NFT, SP and neuronal loss represent a stereotypic central nervous system response to insult and determining what types of insults can lead to the appearance of these classic markers would greatly improve our understanding of the neuropathology of AD.

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# Cerebral Biopsy in the Neurochemical Study of Alzheimer Disease

Andrew W. Procter[1,2] and David M. Bowen[2]
[1] *Dept. of Neurochemistry, Inst. of Neurology, London, U.K.* and
[2] *Dept. of Psychiatry, U.M.D.S. Guy's Hospital Campus, London, U.K.*

## INTRODUCTION

It is generally considered that the diagnosis of Alzheimer's Disease (AD) can only be made with clinical evidence of dementia during life and the histological demonstration of the pathological changes in brain tissue obtained either at autopsy (McKhann, 1984; MRC, 1987) or diagnostic craniotomy (McKhann, 1984). The biochemical examination of the brain is not necessary for this diagnosis to be made, given the present understanding of the condition. Biochemical studies do however provide additional information about pathology - by identifying neurotransmitter systems susceptible to damage; and about etiology - by the study of fundamental cellular mechanisms which may be abnormal in the condition. Unequivocal pathognomonic biochemical changes have not yet been identified, but probably the closest to such change is the loss of cholinergic innervation of the brain. However doubts have been raised about the invariance of its occurrence in the condition, based on choline acetyltransferase (ChAT) specific activity measurements (Palmer et al, 1986).

Thus at present, although the biochemical examination of brain tissue obtained at cerebral biopsy does not in itself provide a diagnostic technique for dementia, this is not the case for the histological examination of such tissue which is an established technique for the investigation of neurodegenerative conditions (Sim et al, 1966). The biochemical study of this tissue provides a unique research opportunity, which may eventually provide diagnostic information. However direct comparison with the more usual post-mortem studies is not possible and the interpretation of both biopsy and post-mortem data must take account of many factors which will be discussed below. Table 1 shows that even in undemented control subjects there are significant differences of between biochemical markers of some neurotransmitter systems assessed post-mortem and ante-mortem, using samples removed at neurosurgery.

Table 1. Comparison of Some Neurochemical Measures in the Temporal Cortex Obtained Ante-mortem and Post-mortem from Undemented Subjects

| | Ante-mortem | Post-mortem |
|---|---|---|
| Measures Unchanged | | |
| Chat activity (pmol/min) | 91 ± 8 (12) | 110 ± 9 (13) |
| Measures Reduced | | |
| 5HT (pmol) | 5.9 ± 0.7 (15) | 1.8 ± 0.4 (8)* |
| NA (pmol) | 1.8 ± 0.1 (14) | 0.9 ± 0.1 (8)* |
| DA (pmol) | 1.3 ± 0.1 (14) | 0.4 ± 0.2 (8)* |
| SLIR (pmol) | 1.4 ± 0.3 (8) | 0.4 ± 0.1 (5)* |
| PAG activity (pmol/min) | 11.4 ± 0.6 (6) | 5.5 ± 1.5 (8)* |
| Measures Increased | | |
| Asp (nmol) | 17.1 ± 0.7 (10) | 29.9 ± 3.4 (8)* |
| Glu (nmol) | 118.4 ± 5.3 (14) | 137.1 ± 5.5 (8)* |
| GABA (nmol) | 19.3 ± 1.0 (10) | 26.1 ± 2.4 (8)* |

Subjects were selected so that mean ages of both groups were similar. Determinations were made as described elsewhere (Francis et al, 1987; Palmer et al, 1987a,b,c; Procter et al, 1988c). Values are mean ± SEM (n) per mg protein. ChAT, choline acetyltransferase; 5HT, serotonin; NA, noradrenaline; DA, dopamine; SLIR, somatostatin-like immunoreactivity; PAG, phosphate-activated glutaminase; Asp, aspartic acid; glu, glutamic acid; GABA, gamma aminobutyric acid. * indicates significantly different from ante-mortem values ($p < 0.05$, Mann-Whitney U test, two-tailed).

Table 2 shows that comparisons made between AD and control subjects on the basis of biopsy or autopsy material may not always agree. Post-mortem changes are only some of the factors which may contribute to this. Biochemical studies are usually made on small samples (in epidemiological terms) and the selection of subjects for such studies is influenced by many, unspecified factors, some of which will be discussed in the remainder of this chapter.

There are aspects of the disease process which influence the biochemical determinations which cannot be taken into account by biopsy studies. These too will be discussed, and emphasise that it is an oversimplification to consider that a biopsy study of AD provides the same information as a post-mortem study merely free of post-mortem effects. Many of the factors discussed here are relevant to the study of other brain disorders, albeit that few are amenable to study by "biopsy".

Table 2. Summary of Studies of Neurochemical Measures in Alzheimer's Disease Made on Temporal Cortex Obtained by Neurosurgery or Prompt Autopsy.

| | AD as percent of control | |
|---|---|---|
| Measure | Neurosurgery | Autopsy |
| 5HT | 31[3] | 27[4] |
| Asp | 124[4] | ns[4] |
| Glu | 86[4] | 66[4] |
| GABA | ns[2] | 76[2] |
| SLIR | ns[1] | 67[2] |
| PAG activity | ns[4] | ns[4] |
| ChAT activity | 35[3] | 22[4] |

Post-mortem and neurosurgical samples were processed identically on removal from the skull. The mean interval from emergence of symptoms to neurosurgery was 2 - 4 years, and to autopsy was 8 - 11 years. Percentages are given where a measure in AD was significantly different from control ($p < 0.05$). Superscripts refer to individual studies; [1] Francis et al, 1987; [2] Lowe et al, 1988; [3] Palmer et al, 1987c; [4] Procter et al, 1988c. ns, not significant. Other abbreviations as Table 1.

## FACTORS INFLUENCING BIOCHEMICAL STUDIES

### Selection of Patients

This consideration applies equally to biopsy and post-mortem studies. Only a small proportion of all elderly demented patients are examined at autopsy. In the United Kingdom those who die at home are rarely so examined, thus it seems likely that post-mortem series will be subject to the unintentional selection of cases for which institutional (especially hospital) care was necessary. In the United Kingdom the estimated prevalence of dementia in persons over the age of 65 years is in the order of 3% (Lindesay et al, 1989) which means that there are about a quarter of a million sufferers (based on the 1981 census figures), yet the number of diagnostic biopsies performed for this condition is unlikely to exceed 50 per year.

Factors which are likely to influence the selection of patients for post-mortem examination or cerebral biopsy will be considered.

Age and social class The social consequences of dementia are determined in part by the patients age and social background. Thus a young patient in non-manual employment and therefore very dependent upon his or her intellectual functioning, seems likely to come to medical attention sooner than a retired person with access to social services support.

Referral and investigation patterns differ between young and elderly patients, in part because of different societal demands upon, and expectations of, the two groups (see Slater and Gearing, 1988).

Clinical features Although sociodemographic factors do play a role in determining referral and investigation patterns, the clinical features of the disease probably have as great a role, and may be of importance to the biochemist. Much attention has been paid to the cognitive symptoms of AD, however cognitive impairment is frequently associated with behavioural and personality changes. These appear to have been overlooked in recent years (but see Rosen et al, 1984; Fairburn and Hope, 1988) even though the original case described by Alzheimer (Wilkins and Brody, 1969) developed such symptoms in the form of "jealousy towards her husband" as the first noticeable sign of the disease before loss of memory. These behavioural changes are often a source of distress to carers and may determine whether the patients come to medical attention and the care they subsequently require (Sanford, 1975; Chenoweth and Spencer, 1986; see also Procter and Bowen, 1988). Thus biochemical studies of patients previously resident in institutions may be subject to the inadvertant selection of cases with prominent behavioural symptoms.

That this may have an influence upon biochemical determinations is illustrated by the example of aggression, a frequently encountered symptom. Determinations have been made of serotonin (5-HT) concentrations and 5-$HT_2$ recognition sites in frontal cortex obtained at post-mortem from 16 patients with AD and appropriate control subjects. Retrospective review of the case notes provided an assessment of the presence of prominent behavioural symptoms (aggression, depression and wandering), all as described previously (Procter et al, 1988a; Palmer 1988b). Table 3 shows that loss of these biochemical measures was confined to those subjects with prominent aggression in life, although the analysis of the group as a whole appears to indicate that loss of the recognition site is a general feature of AD. Thus the prominent disorder of some markers of serotonin innervation in AD (Palmer et al, 1987a; Procter et al, 1988a,b) may in fact be confined to a subgroup which is over-represented in post-mortem studies.

Sociodemographic and clinical factors must be considered if studies are to be representative of AD generally and may contribute to the differences between biopsy and post-mortem studies. However they are often difficult to quantify - particularly behavioural disorder (see Ward et al, 1989).

Stage of Disease

It is slightly easier to take account of the stage of the disease at which the tissue is examined. While AD is a progressive disease, this is rarely acknowledged in the interpretation of post-mortem biochemical studies. Cerebral biopsy is a diagnostic procedure and is therefore performed soon after the onset of symptoms. Post-mortem examination is made at the inevitable end-point of the disease, death often several years later (Footnote Table 2). The clinical manifestation of the progression of the disease is a

Table 3. Serotonin Concentration and Type-2 Recognition site in Orbital Gyrus Post-mortem from AD and Control Subjects Unselected and Also Subdivided According to Presence of Aggressive Behaviour

| | 5HT concentration (pmol/mg protein) | $5HT_2$ site (fmol/mg protein) |
|---|---|---|
| Control subjects | 0.74 ± 0.11 (14)[a] | 20.7 ± 2.4 (13)[c,e] |
| AD subjects | | |
| All | 0.57 ± 0.06 (17) | 11.8 ± 1.3 (16)[c] |
| Aggressive | 0.40 ± 0.06 (4)[a,b] | 6.2 ± 0.9 (4)[d,e] |
| Others | 0.62 ± 0.06 (13)[b] | 13.7 ± 1.3 (12)[d] |

Results are mean ± SEM (n). 5HT concentration was assayed by HPLC as described previously (Palmer et al, 1987a). $5HT_2$ recognition site was assayed using [$^3$H]-ketanserin at 5 nM, as described elsewhere (Procter et al, 1988a). Superscript letters indicate pairs of results significantly different from each other; $^{a,b}p < 0.05$, $^{c,d,e}p < 0.02$, Mann-Whitney U-test, two-tailed.

worsening of the symptoms; the histological and biochemical concomitants of this are open to speculation, as such examination is usually only made at one stage of the disease (i.e. after death). However it seems likely that the worsening of the dementia is associated with more severe pathology and more widespread changes (see Pearson et al, 1985; Pearson and Powell, 1989). This may explain the apparent discrepancy between biochemical markers of cortical gamma-aminobutyric acid (GABA) innervation of the cortex determined in biopsy tissue and at post-mortem (Lowe et al, 1988; also for review). Several studies have shown profound loss of GABA in post-mortem tissue, especially when severe cases have been selected, but this is not the case for determinations on biopsy tissue obtained early in the disease (Table 4).

Peri-mortem Epiphenomena

Some biochemical measures of GABA innervation also illustrate the influences of peri-mortem epiphenomena, from which biopsy tissue is notably free.

Agonal effects Like many biochemical determinations in human brain, the activity of the enzyme for the synthesis of GABA, glutamic acid decarboxylase (GAD), is markedly affected by the manner of the patients death. Thus low activity of the enzyme is present in the brains of patients dying with protracted illnesses (Bowen et al, 1976).

Table 4. Summary of Studies of GABA Innervation of the Middle and/or Superior Temporal Gyrus in Alzheimer's Disease and Control Ante-mortem and Post-mortem.

| | AD as percentage of control | |
|---|---|---|
| Marker | Ante-mortem | Post mortem |
| GAD Activity | ns[1] | agonal state effects[a] |
| GABA Release | ns[2] | nd[a] |
| GABA Content | ns[3] | 58[4,b]<br>70[5]<br>71[3]<br>72[6] |

Percentages are given where a measure in AD was significantly different from control ($p < 0.05$). Superscript numbers refer to individual studies; [1] Spillane et al, 1977; [2] Smith et al, 1983; [3] Lowe et al, 1988; [4] Ellison et al, 1986; [5] Arai et al, 1984; [6] Rossor et al, 1984. ns, not significant. nd, not determined. [a] see "Agonal effects" text; [b] indicates a study in which deliberate selection of severe cases was made.

Patients with AD tend to die in this manner, typically by bronchopneumonia, and allowance for the influence of this must be made if valid conclusions about disease related changes are to be drawn. This can also be illustrated by the study of the regulation by glycine of the N-methyl-D-aspartate receptor/ionophore complex. In common with the situation in experimental animals (Reynolds et al, 1987; Wong et al, 1987), the binding of the radioligands [$^3$H]-MK-801 ([$^3$H]-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate) and [$^3$H]-TCP ([$^3$H]-thienyl cyclohexyl-piperidine) to the ionophore of the NMDA receptor complex is regulated by glutamic acid and glycine in a synergistic manner (Procter et al, 1989a,b). In control subjects, the extent to which glycine will enhance this binding is dependent upon the mode of the patients death (Table 5). The difference between AD and control determinations post-mortem may be accentuated by this phenomenon as few of the AD subjects died a sudden death. However a disease related difference may still be observed in subjects studied with cerebral biopsy free from peri-mortem influences (Table 5), and in post-mortem tissue when Scatchard analysis is performed on 4 control and 4 AD subjects matched for mode of death, age and post-mortem delay. For these samples $K_D$ values (mean $\pm$ SEM) were 34.4 $\pm$ 4.6 nM and 62.2 $\pm$ 3.9 nM respectively ($p < 0.05$ Mann-Whitney U test, two tailed; Procter et al, 1989b).

Table 5. [$^3$H]-MK-801 Binding in Cerebral Cortex From Control and Alzheimer Subjects

| | [$^3$H]-MK-801 Binding (fmol/mg protein) | |
|---|---|---|
| | With 30 uM glu | With 30 uM glu and 3 uM gly |
| POST MORTEM | | |
| Control subjects | | |
| All (n=16) | 102.2 ± 6.0 | 231.4 ± 14.2[a] |
| Sudden death (n=9) | 101.9 ± 6.3 | 256.9 ± 6.2[b] |
| Protracted death (n=7) | 102.6 ± 11.7 | 198.7 ± 6.0[b] |
| Alzheimer's Disease | | |
| All (n=14) | 100.6 ± 5.3 | 170.6 ± 12.1[a] |
| Sudden death (n=2) | 105.0, 118.0 | 218.0, 198.0 |
| Protracted death (n=12) | 98.8 ± 6.0 | 164.3 ± 13.2 |
| ANTE-MORTEM | | |
| Control subjects (n=23) | nd | 655.5 ± 31.0[c] |
| Alzheimer's Disease (n=7) | nd | 496.4 ± 51.0[c] |

Well washed membranes were prepared from superior frontal cortex of AD and control autopsy, AD biopsy (3 temporal and 4 frontal cortex) and neurosurgical control (6 temporal and 17 frontal cortex) samples. Determinations of specific binding of [$^3$H]-MK-801 were made in triplicate in 5 mM TRIS-HCl pH 7.4 with added glutamic acid (glu) in the presence and absence of added glycine (gly). Mixtures were incubated at 25°C before being subject to rapid filtration, washing with ice-cold buffer and liquid scintillation spectrometry. Post-mortem determinations were made using 5 nM [$^3$H]-MK-801, and ante-mortem using 10 nM as described elsewhere (Procter et al, 1989a,b). Values are mean ± SEM, except for AD sudden death, where individual values are shown. Values with the same superscript letter are significantly different: [a] $p < 0.01$, [b] $p < 0.05$, [c] $p < 0.05$; Mann-Whitney U-test, two-tailed. nd, not determined.

The reliable assessment of the length of pre-terminal coma is often difficult to make retrospectively from routine clinical records as these may be incomplete or do not contain the information relevant to research. A more reliable method is to assess each patient's level of consciousness routinely each day, so that these records may be used after death to give a prospective assessment of terminal coma.

The exact nature of the peri-mortem influences which cause this phenomenon is not known, but the effect of hypoxia/ischaemia are likely to be of major importance, and are amenable to study in experimental animals.

Related and possibly augmenting the effects of pre-mortem coma, are post-mortem changes. The interval from death to autopsy is known to influence many biochemical determinations (Palmer et al, 1988a; Table 1). Particularly sensitive appear to be complex processes such as the release of neurotransmitters from preparations containing synaptosomes (Procter et al, 1988c) and the nucleic acids, where special precautions are required (Schmechel et al, 1988). As is also true for experimental animals, it is possible to demonstrate release of neurotransmitters including glutamic acid, aspartic acid, GABA, and dopamine from human brain tissue removed neurosurgically. Similar preparations may be made of fresh (i.e. unfrozen) tissue obtained very promptly after death (less than 2 hours), however it is not possible to demonstrate similar release under even these conditions regardless of the nature of the disorder from which patients had suffered before death (Procter et al 1988c).

Handling of tissue That the absence of calcium-dependent release of neurotransmitters described above was a feature of unfrozen post-mortem tissue, processed in the same way as biopsy material, excludes the influence of another source of systematic variability between some studies. The manner in which the tissue is handled after removal from the skull, either by neurosurgery or at autopsy, affects the activity of many measures. An apparently valid and fairly reliable measure of glutamatergic nerve endings in brain tissue is the uptake of [$^3$H]-D-aspartic acid into preparations of synaptosomes. Two methods have been proposed for the application of this technique to human brain post-mortem. One (Procter et al, 1988c) involves obtaining tissue promptly after death, processing it fresh to prepare tissue prisms and making determinations upon these immediately. The other (Hardy et al, 1987) involves a subcellular fractionation step to yield a preparation enriched in synaptosomes which is subsequently frozen before determinations are made. The use of tissue prisms, a preparation shown to be composed predominantly of intact nerve endings when examined by electron microscopy (Sims et al, 1981), minimises the possibility of the production of inappropriate subfractions when diseased tissue is studied. Similarly the freezing of the preparation, which may be associated with a loss of activity of 50% of this marker in experimental animals (Hardy et al, 1987), is best avoided wherever possible as the degree of differential susceptibility to freezing in disease states is unknown.

Other influences The influence of co-existing medical conditions and drugs for their treatment must always be considered - in both post-mortem and biopsy studies. However the patients considered for investigation by biopsy are usually so selected because they are conspicuously free from any other illness. All patients, both control and demented, examined by biopsy are subject to a general anaesthetic, but other drugs which they receive may be different. Treatment with psychotropic drugs appears to affect some biochemical measurements (Procter et al, 1988a), but patients with AD do not seem to be subject to any systematic effects of anaesthetics (Sims et al, 1981).

Control Groups

The importance of control groups, matched for factors considered above cannot be over emphasised. For most post-mortem studies such subjects are available from most diligent pathology departments. However the prospective assessment of control subjects who may be examined promptly post-mortem may be more difficult as such subjects must die with ready access of pathology and biochemistry services. The majority of potential subjects are resident in long stay institutions and hospitals, often because of co-existing psychiatric or neurological disorders, the former of these requiring prolonged drug treatment. One approach to this problem has been the use of a "demented control" group suffering conditions other than AD causing dementia (Procter et al 1988c). These subjects are thus very closely matched to the AD groups, and any differences observed can be attributed with some degree of confidence to AD rather than the varied causes of dementia in the other group.

The selection of control groups for biopsy studies is more difficult, as subjects only undergo craniotomy for major central nervous system disorders. The use of apparently normal tissue removed by neurosurgeons to gain access to deep seated tumours as a normal and necessary part of the procedure offers an ethically and scientifically sound approach. Such tissue macroscopically free from tumour and distant to the site of operative procedures such as diathermy, shares many biochemical properties similar to those seen in tissue removed from experimental animals. These include the $Ca^{2+}$-dependent $K^{+}$-evoked release of neurotransmitters (Procter et al, 1988c), active uptake of neurotransmitter analogues (Procter et al, 1988c), and intact measures of metabolic function (Bowen et al, 1982b). The type of tumour for which the operation was performed does not appear to affect the biochemical activity, (e.g. Sims et al, 1983; Francis et al, 1987; Lowe et al, 1988). However the possibility of microscopic infiltration, or distant metabolic or mechanical interference with function cannot be completely excluded.

## FACTORS WHICH BIOPSY CANNOT ADDRESS

Distribution of lesions

It is impossible for cerebral biopsy to examine more than a very limited area of the cerebral cortex, and certain areas of cortex cannot be biopsied because of the neurological deficits which the patient would suffer as a result. Cortical biopsy studies of patients with cerebral atrophy (Sim et al, 1966; Coblentz et al, 1973, Bowen et al, 1982a) indicate that characteristic neuropathological changes of AD were present in the tissue from the majority of patients, yet a significant minority showed no specific features and a diagnosis of Pick's Disease could be made rarely. It could be argued that the absence of pathological findings simply reflects a relative sparing of the part of the brain sampled by the biopsy, and positive AD pathology would have been found had a different area been sampled (Sim et al, 1966). An alternative explanation is that dementia and cerebral atrophy may encompass a more heterogeneous group of disorders than is often acknowledged (Bowen and Neary, 1985).

Post-mortem (e.g. Brun, 1983; Esiri et al, 1989; see also Pearson et al 1985; Rogers and Morrison, 1985; Procter et al 1988a) and in vivo (e.g. Neary et al, 1987) studies of AD indicate that certain areas of the brain are more susceptible to damage than others. It has been suggested that the distribution of this pathology reflects the known anatomical organization of the cerebral cortex (Pearson et al, 1985), but it is unknown whether the pathology develops sequentially or simultaneously in these areas as examination of the entire brain from one patient can only be made once (i.e. at death). Cerebral biopsy can only examine a small area of brain , and in vivo imaging techniques are indirect and may be complicated by the effect of regional cerebral atrophy (see below).

Thus a very limited understanding of the biochemistry of AD can be obtained by biopsy. It is not possible to examine deep structures such as the amygdala, which may be of importance in the early stages of the disease where lesions may contribute to the production of early behavioural symptoms (Procter, 1988) and loss of cholinergic innervation may occur in the absence of gross loss in the neocortex (Palmer et al, 1986).

Regional Atrophy

Loss of brain tissue is a prominent macroscopic change in AD, and if grey matter from anatomically defined areas is dissected and weighed it can be shown that this loss is not uniform throughout the cortex (Najlerahim and Bowen, 1988). The cerebral atrophy shows a similar pattern to the both the microscopic (Brun, 1983; Esiri et al, 1989) and biochemical (Procter et al, 1988a,b) pathology, the parietal and temporal lobes being particularly affected. Which cellular elements are lost in this process is not known because the practice of reporting biochemical results relative to unit mass (e.g. per mg of protein) does not make allowance for any reduction in volume of brain structures. Shrinkage or loss of some structures without change of others may lead to the reporting of an increase in the indices of the unaffected structure and perhaps more importantly an apparently small decrease in the markers of affected structures. Loss of a marker may be considerably underestimated should the structure to which it relates represent a large proportion of the tissue volume, such as pyramidal neurones in the cerebral cortex. Similarly should loss occur equally of all structures (e.g. of pyramidal neurones and associated structures organised in columns) there may be no change reported even in severely atrophied tissue (Procter et al, 1989a; Francis and Bowen, 1989).

This issue may be addressed by the dissection of entire regions of the brain, and expressing biochemical data relative to the protein content, not of the small sample of grey matter examined, but of the entire brain region. In this way loss of pyramidal cells from temporal and parietal cortex has been indicated, as well as more widespread loss of a subpopulation of interneurones from the frontal cortex (Bowen et al, 1989b).

## FUTURE DIRECTIONS FOR BIOPSY RESEARCH

Although cerebral biopsy offers the only technique for the definitive diagnosis of AD in life (McKhann et al, 1984), and is associated with few complications (Neary et al, 1986b), it has nonetheless had limited application in routine clinical practice. In part this is probably because of the invasive nature of the technique and the need for a general anaesthetic. The recent demonstration of abnormal proteins in cells of the olfactory mucosa post-mortem in AD (Talamo et al, 1988) is, given the limitations of post-mortem studies discussed above, of theoretical interest (Pearson and Powell, 1989) and also indicates a potential, less invasive diagnostic procedure. The olfactory mucosa can be biopsied without the need for a general anaesthetic (Douek et al, 1975), and yet contains cells derived from the brain, albeit with some unusual features. Such tissue may be of value for investigating metabolic properties of neurones which may be abnormal in AD (Procter et al, 1988c).

## CONCLUSIONS

Histological studies of cerebral biopsy in AD have identified a disorder of pyramidal cells in the cerebral cortex (Neary et al, 1986a,c).

Biochemical studies of ascending innervation of such tissue indicates a prominent cholinergic deficit (Sims et al, 1983) which has been taken as the basis for a rational pharmacological treatment of symptoms. Markers of serotonergic fibres are also lost from the cortex (Palmer et al,1987a), although any influence of unintentional selection of cases because of behavioural symptoms has not been addressed.

Measures of interneurones including concentrations of GABA and SLIR tend to be lost at a late stage of the disease, especially in severely affected cases. This is not a feature of biopsy material obtained earlier in the course of the disease (Francis et al, 1987; Lowe et al, 1988).

The biochemical assessment of pyramidal neurones is difficult. This is in part because the likely transmitter candidate of these cells, glutamic or aspartic acid, is widely distributed and measurements may reflect not only degrees of denervation, but also more generalised metabolic dysfunction in AD, as well as the influence of epiphenomena (Procter et al, 1988c). A further complication is the effect of cerebral atrophy on such biochemical measures conventionally reported (see "Regional cerebral atrophy" above).

Apparently independent of these influences however, is the disease related reduction in efficacy of glycine at promoting binding of the NMDA-receptor-ionophore associated ligands MK-801 and TCP which probably occur at an early stage of the disease (Table 5). It seems likely that the majority of synapses pyramidal cells make are with other pyramidal cells (see Procter et al, 1988c), and this may therefore reflect a functional rather than structural abnormality of these cells. The effect of this on cytosolic calcium concentrations may explain their particular vulnerability in AD, and a high priority must be the evaluation of the

therapeutic potential of drugs active at the glycine modulatory domain (Bowen et al, 1989a). Possibly by virtue of its effect on the NMDA-receptor/ionophore complex, MK-801 causes pathology in a sub-population of pyramidal cells in the posterior cingulate gyrus (Olney et al, 1989), a region particularly vulnerable in AD (Brun, 1983).

Although cortical tissue from patients with deep-seated neoplastic lesions seems suitable as a control group, the use of such material has been questioned (Tarbit et al, 1981; Sherwin et al, 1988). Therefore in a recent study (Lowe et al, 1989) two additional groups were used and the content of free amino acids determined. Of the abundant ones, aspartate, glutamine and serine, showed significant differences in concentration between subgroups of the non-demented subjects. Values for the other compounds in neocortex removed for access to deep-seated neoplastic lesions were taken as an indication of values for normal tissue. Content of the other abundant amino acids were in AD, either unaltered (GABA, glycine and taurine), increased (alanine), or decreased (glutamate). Content of the latter for individual AD samples from the mid-temporal gyrus shows a correlation with pyramidal neurone count in cortical layer III.

Shrinkage or loss of cortico-cortical association fibres is suspected in AD, principally from the temporal and parietal lobes (Pearson et al, 1985; Procter et al, 1989a). These neurochemical data provide novel evidence that these fibres are glutamatergic. They also appear to identify another specific system of synaptic transmission at which potential therapeutic compounds should be directed. Direct agonism of glutamate recognition sites has been suggested (Deutsch and Morihisa, 1988), but drugs active at the modulatory domains, including that for polyamines (Carter et al, 1989) are potentially less dangerous.

ACKNOWLEDGEMENTS

The authors wish to thank the many people involved in the collection, classification and assay of the samples which have been discussed in this chapter. The work was supported by the Brain Research Trust and Astra Alab.

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# Part 8

# Brain Imaging



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# CT, MRI, and NMR Spectroscopy in Alzheimer Disease*

Liane J. Leedom[1] and Bruce L. Miller[2]
[1] Dept. of Psychiatry, [2] Dept. of Neurology, Harbor-UCLA Medical Center, Los Angeles, CA

## Introduction

Over the past 15 years there have been dramatic improvements in the diagnosis of Alzheimer disease (AD). Two studies done between 1975 and 1979 demonstrated that 30%-50% of patients diagnosed with AD were ultimately discovered to have a different illness (Nott and Fleminger, 1975) (Toone et al., 1979). By 1989 misdiagnosis had dropped below 20% (Tierney et al., 1988) and in many institutions was approximately 10% (Joachim et al., 1986). Although some of this refinement in diagnosis was due to better recognition of the clinical features of AD (Cummings and Benson, 1983), another major factor was the development and easy accessibility of computerized axial tomography (CT). With CT widely available, it became unusual for a patient in the United States to die from a structural brain lesion misdiagnosed as AD. However, CT has not been as definitive for diagnosis as was originally hoped and strategies that attempted to utilize atrophy on CT as a definitive diagnostic test for AD have largely failed.

In the mid 1980s MRI became available and this technique added new refinements in diagnosis and also new problems. MRI gave excellent resolution of brain structure and has been particularly useful in demonstrating lesions in the brainstem and subcortical white matter that could not be seen with CT. Both CT and MRI have been sensitive at defining non-Alzheimer brain injury yet neither technique has lead to a definitive diagnosis of AD. In addition, it has not been possible to utilize either technique to help understand the pathogenesis of AD. However, with the newest of these technologies, in vivo Nuclear Magnetic Resonance (NMR) spectroscopy, there are potential opportunities for improvement in diagnosis and better understanding of the pathogenesis of AD.

This chapter evaluates the applications and limitations of CT and MRI for the diagnosis of AD. First, we describe the use of CT and MRI to rule out non-AD brain diseases and the attempts to quantify brain atrophy and density for the diagnosis of AD. In addition, there is discussion of the significance of the white matter lesions seen with MRI in the brains of many individuals with AD. Finally, there is a brief review of the new technique of NMR spectroscopy and a discussion of its potential for diagnosis in AD.

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*This work was supported by a Physician-Scientist-Award from the National Institute of Aging USPHS A K11 G 00284 and grants from the Department of Mental Health and the Alzheimer Branch form the

The Use of CT and MRI to Rule out Treatable Lesions

CT was a major advance over previous imaging techniques in the diagnosis of treatable causes for dementia. In previous decades, pneumoencephalography, angiography or even exploratory surgery were the only ways to rule out treatable causes for dementia. The exact percentage of patients in whom a treatable cause for dementia will be found is debatable, although in the pre-CT era Marsden found that "10 to 15% of patients had a disease process that could be reversed or halted by appropriate treatment." (Marsden and Harrison, 1972). This study estimated that 10% of patients had multiple strokes, 5% had brain tumors, and 6% had normal pressure hydrocephalus. All of these disease entities could be diagnosed by CT. Similarly, in a CT study of 500 consecutive patients presenting with new onset dementia, Bradshaw et al. (1983), reported that 10% had a treatable lesion.

These figures are probably higher than what is now seen by most neurologists and psychiatrists, although this change is probably due, in part, to the easy availability of CT to primary care physicians. We believe that a dementia work-up is not complete without a CT or MRI. As was emphasized at the National Institute of Health, Consensus Conference on "Differential Diagnosis of Dementing Diseases" (1987), once a history, physical examination and simple laboratory screen have been completed and a cause for dementia has not been elucidated, a CT scan should be performed to rule out a structural brain abnormality.

Cerebral Atrophy on CT and the Diagnosis of Alzheimer Disease

Because AD is pathologically characterized by brain atrophy and neuronal cell loss it was hypothesized that atrophy on CT would correlate with the degree dementia and that the presence of atrophy would distinguish AD patients from those suffering from treatable dementia. Prior to CT, clinicians relied heavily on pneumoencephalography for the evaluation of atrophy in suspected dementia. This technique proved unsatisfactory because it carried with it significant morbidity. CT has proven to be more accurate in the diagnosis of atrophy than pneumoencephalography and without the attendant risks (Roberts et al., 1976).

Beginning with the first studies of dementia with CT, during the mid 1970's, investigators developed a variety of techniques to quantify atrophy. In this chapter atrophy will be defined as an increase of cerebroventricular size, sulcal widening and/or a combination of the two. Specific techniques to measure atrophy will be briefly described here and are graphically outlined in Figure 1 (on the following page).

The first methods to quantify atrophy utilized simple ruler assisted measurements of ventricular width (so-called linear measurements) and ignored brain and cranial size. However, it was quickly noted that the simple measurement of ventricular width was unsatisfactory because it failed to control for head size which influenced ventricular size. For this reason linear measures of ventricular to cranial width were adopted and utilized to estimate brain atrophy (Earnest et al., 1979) (Damasio et al., 1983). As can be seen by comparing Figures 1B and 1C, simple linear measures of ventricular width can vary according to where the ventricle is measured so to avoid this

source of error investigators turned to planimetric measurements of ventricular area. For example, as is shown in Figure 1C, the ventricular and brain areas would be traced and measured and the ratio expressed as the ventricular to brain ratio or VB (Synek and Reuben, 1976). A more recent refinement of this technique has been to measure ventricular and brain size from all of the sections (Figures 1A, 1B and 1C) and to sum this total to derive an estimate of total brain and ventricular volume. Studies that utilized linear parameters will be described first followed by area and then volumetric studies.

Figure 1

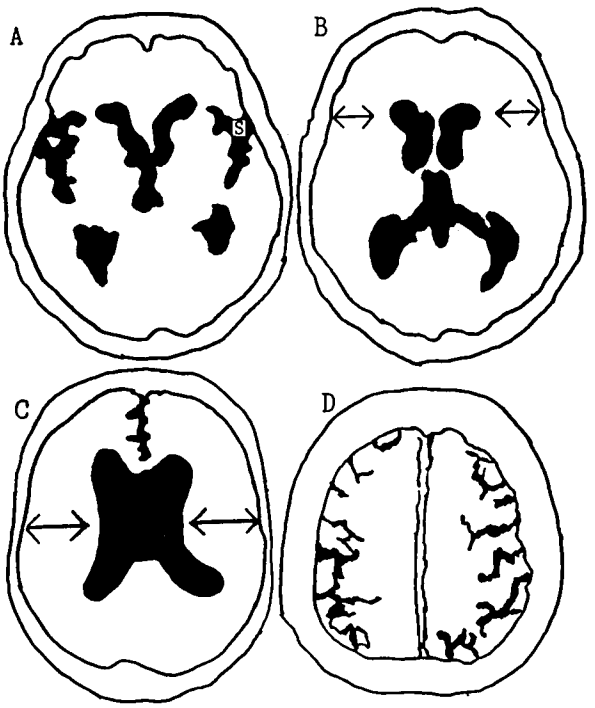



Figure 1: This demonstrates 4 CT slices that can be used to measure atrophy. A. The sylvian fissures are marked S. B. The frontal horns of the lateral ventricles are the black areas in the center of the scan. C. The lateral ventricles above the level of the caudate nucleus appear as black. Regions marked by arrows in B and C are the areas where linear measurements are made. D. This shows the cortex above the lateral ventricles. This slice is utilized to measure sulcal width.

Early studies made use of polaroid pictures of cathode ray images to quantify atrophy and translucent rulers were used to measure brain structures such as the frontal horns, lateral ventricles and cortical sulci. On the basis of these measurements, criteria were developed for diagnosing atrophy. In one of the first studies utilizing linear methods, Huckman et al. (1975) noted that 60% of "normal" individuals 65 years of age or older were classified as having questionable to severe atrophy

(i.e. false positive tests). Interestingly however, only 3% of documented dementia patients had normal scans. Two patients in the study were later discovered to have a treatable cause for dementia. Both of these cases were diagnosed with "questionable atrophy." The authors hypothesized that patients with a dementia and a non-atrophic CT scan were more likely to have a treatable dementia than those with severe atrophy. However, as was noted by Ford and Winter (1982), "CT findings of cortical atrophy should not be accepted as evidence of senile dementia."

The observation that 60% of "normal" individuals over 65 had some brain atrophy demonstrated the need to investigate the effect of age on atrophic changes. Kaszniak et al. (1979) studied the relationship between atrophy and age in a demented population and found a modest though significant correlation. Hughes and Gado (1981) also found that linear ventricular and sulcal measures correlated with age in both demented and non-demented subjects. This corroborated findings of Barron et al. (1976) in normals and demonstrated that because cerebral atrophy occurred in normal elderly, studies measuring atrophy in demented patients needed to control for the effects of normal aging.

As with simple linear measures, measurements of ventricular area increase with age (Roberts and Caird, 1976). In one study age-corrected ventricle-brain ratios were abnormal in half of dementia patients and only one of thirty control subjects (Brinkman et al., 1981). Damasio et al. (1983) found that using this method for determining atrophy led to a correct identification of 89% of normals and 78% of demented subjects respectively. Despite the use of an elegant discriminant analysis with special weighting for various features of atrophy, the false positive rate was 11% and the false negative rate 22%. These high percentages of false negatives and false positives point out the difficulty in using atrophy as a way of either ruling in or ruling out AD.

Yamaura et al. (1980) measured the percent volume of the cranial cavity occupied by brain tissue and CSF respectively. This study of normals revealed that the volume occupied by CSF was proportionally low in children and adolescents, reached a plateau in 20-49 year olds and then increased after age 50. Ventricular volumes of normals over 80 were the largest. Gado et al. (1982) examined volumetric measurements of CSF spaces in presumed Alzheimer's disease and control subjects. In this study volumetric measures highly correlated with linear measures in individual subjects although, volumetric indices separated demented groups from controls better than did linear indices. This finding was later confirmed by Albert et al. (1984).

Other investigators attempted to use the CT density number or Hounsfield unit to quantify the atrophy of AD. The highest density numbers occur in tissues like bone, mid-range numbers for brain and the lowest numbers are seen with cerebrospinal fluid. In a study of patients with Huntington and AD, Naeser et al. (1980) suggested that CT density numbers better differentiated dementia patients from controls than did traditional measures of atrophy. Bondareff et al. (1981) used these numbers to compare patients with AD with normal elderly. They found significantly decreased density numbers in the AD compared to controls, although there was considerable overlap between the two groups.

However, Gado et al. (1982) and Wilson et al. (1982) found no significant relationship between CT density number and AD. Most investigators now believe that CT density numbers by themselves are insensitive measures for diagnosing AD. However, Albert et al. (1984) suggested that the use of volumetric measurements in combination with a CT density number improved discrimination of AD from normal controls with an accuracy of 93.5%.

More recent work has focused on the measurement of atrophy in the temporal lobes (LeMay et al., 1986) (LeMay, 1986). These authors noted that they could differentiate patients with AD from normals with an accuracy of 88.57% using discriminant function analysis that included analysis of temporal regions. Linear measures of atrophy only differentiated 65.20% of the patients from normals. Sandor et al. (1988), using a simple technique of area measurement, correctly differentiated AD patients from normal controls in 94% of cases. The success of this technique was attributed to analysis of atrophy on temporal lobe slices as measured by increased ventricular size. Kido et al. (1989) showed that temporal horns larger than 3mm were not found in any of the 29 normal controls while measurements of this size were found in 23 of 39 AD patients. Although these measurements of temporal atrophy strongly distinguish groups of AD from groups of controls they cannot be used to make a definitive diagnosis in an individual patient.

Other investigators have focused on whether progressive atrophy on serial CT could be used to diagnose AD. In a two-year follow-up study of AD patients, Naguib and Levy (1982) divided subjects according to whether or not the illness had worsened over the follow-up period. The group of patients that deteriorated had a significantly greater increase in atrophy as measured by ventricular-brain area than did the group of patients who did not change. Luxenberg et al. (1987) examined ventricular volume and neuropsychological test scores in 18 AD and 12 control subjects. Control subjects were scanned twice with an average of 1,197 days between scans. The scan interval for AD patients was an average of 508 and 457 days for men and women respectively. In controls, there was no change in lateral ventricular volume between the two scans. In contrast, significant increases in ventricular volume were seen in AD patients. Also, increased ventricular volume significantly correlated with the decline in neuropsychological test scores. One group (de Leon et al., 1989) reported that in a three-year longitudinal study of AD patients and controls that the annual rate of increase in ventricular volume was found to be 9% in AD patients and 2% in control subjects. However, the rate of clinical decline of AD patients poorly correlated with the rate of increase in ventricular volume. Although progressive atrophy occurs in the brains of patients with AD, it will also occur in non-AD dementias and there has been no pathology-confirmed study that these changes are either specific or sensitive for AD.

Finally it appears that atrophy weakly predicts performance on cognitive tasks in AD subjects. Earnest et al. (1979) studied 59 normal individuals over age 60 utilizing standard measures of neuropsychological function and ventricle-brain CT ratios. They found that both atrophy and cognitive performance worsened with increasing age although, atrophy alone was a poor predictor of cognitive performance. This finding was supported in a study of

suspected dementia patients by of Jacoby et al. (1980). Computerized volume measurements also correlated with the degree of dementia in AD patients (Creasey et al., 1986). In summary, while the presence of atrophy does not appear to be related to cognitive performance in normals, the degree of dementia in demented patients is weakly correlated with cerebral atrophy.

Despite a refinement in technology and modest improvements in diagnostic accuracy with volumetric measures, the axiom set forth in the first study (Huckman et al., 1975) of CT in dementia still holds true "not all demented patients have atrophy and not all patients with atrophy are demented."

Summary

Several principles summarize the findings in the use of CT in the diagnostic evaluation of AD subjects.

1. CT is useful in ruling out treatable causes for dementia.
2. Cortical atrophy and concomitant ventricular enlargement, especially in the temporal lobes occurs with AD.
3. The measurement of atrophy will distinguish groups of patients with dementia from groups of normals, although one cannot use the presence of atrophy on a CT scan to make a diagnosis of AD in an individual patient.

White Matter Lesions with CT and MRI in Alzheimer disease

With third generation CT and MRI many elderly individuals including patients with AD have subtle abnormalities in the subcortical white matter. These findings have complicated the interpretation of CT and MRI in the elderly and, even today, there is ongoing controversy about their prevalence, etiology and clinical significance. These three issues will be briefly discussed here as all have relevance to the diagnosis of AD. Although the ability to see white matter lesions has probably complicated the diagnosis of AD, some authors have suggested that these lesions can be utilized to help differentiate AD from multi-infarct dementia (Erkinjuntti et al., 1987).

Bradley et al. (1984) were the first to note that small white matter lesions were present in approximately 30% of all patients over the age of 65. Similarly, in a study on normal aging we found that two-thirds of normal individuals living independently in the community had an area of increased signal on the T2 weighted image in the periventricular or subcortical white matter (unpublished data). The vast majority with lesions had either a small periventricular rim or one to two small punctate lesions (Miller et al., 1989) as is shown in Figure 2. These and other reports suggest that the prevalence of white matter lesions appear to increase in elderly individuals, and in patients with hypertension and/or a history of stroke (Inzitari et al., 1987) (Brant-Zawadki et al., 1985) (Ajax et al., 1986) (Gupta et al., 1988) (Englund et al., 1989).

In our study on normal aging, when white matter lesions were quantified, 12% of the normals had a total lesion burden of more than 2.5 $cm^2$ in size. Some patients had lesions that involved as much as 15 $cm^2$ of the subcortical white matter. A patient with vascular disease and extensive white matter lesions is shown in

Figure 3. None of the patients without large white matter lesions had significant cognitive deficits while 40% of those with lesions greater than 2.5 $cm^1$ had serious neuropsychological deficits. This work suggests that large white matter lesions often, but not always, are associated with cognitive impairment.

Steingart et al. (1987) found large white matter lesions on CT which they called "leukoaraiosis" in 35% of 140 demented patients compared to 10.9% of normal control subjects while Hershey et al. (1987) showed that 88% of demented elderly and 61% of non-demented patients with ischemic vascular disease had at least one white matter lesion on MRI. It is hard to compare these studies because MRI has far greater sensitivity to white matter lesions. In a comparison of MRI and CT in dementia, Johnson et al. (1987) showed that abnormalities in subcortical white matter and hippocampus as well as ventriculomegaly were more evident on MRI than CT although linear ventricular spans did not differ with the two techniques.

Figure 2.

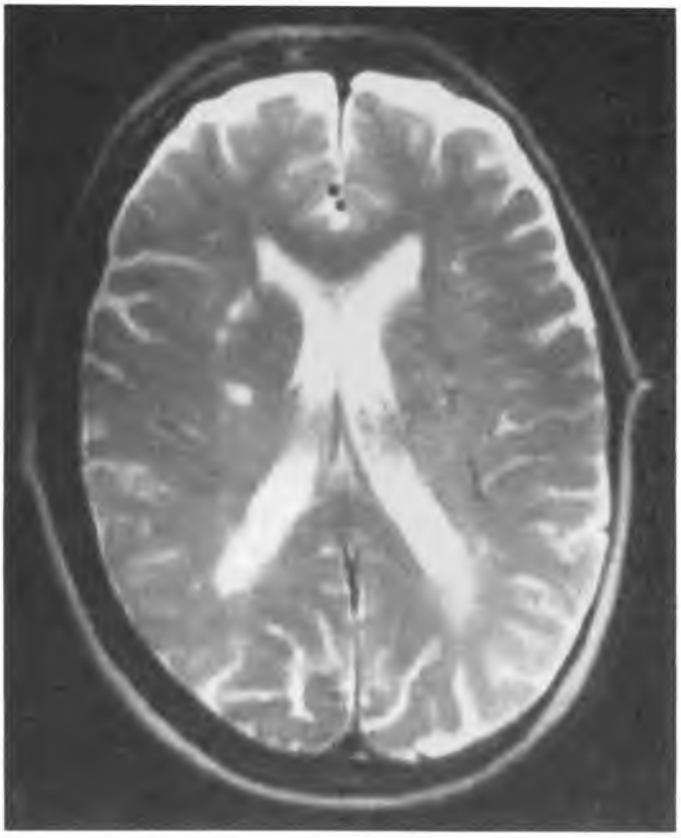


Figure 2: This T2 weighted transaxial MRI of a normal elderly subject shows three small areas of hyperintesity in subcortical white matter and a small rim of periventricular hyperintensity.

Figure 3.

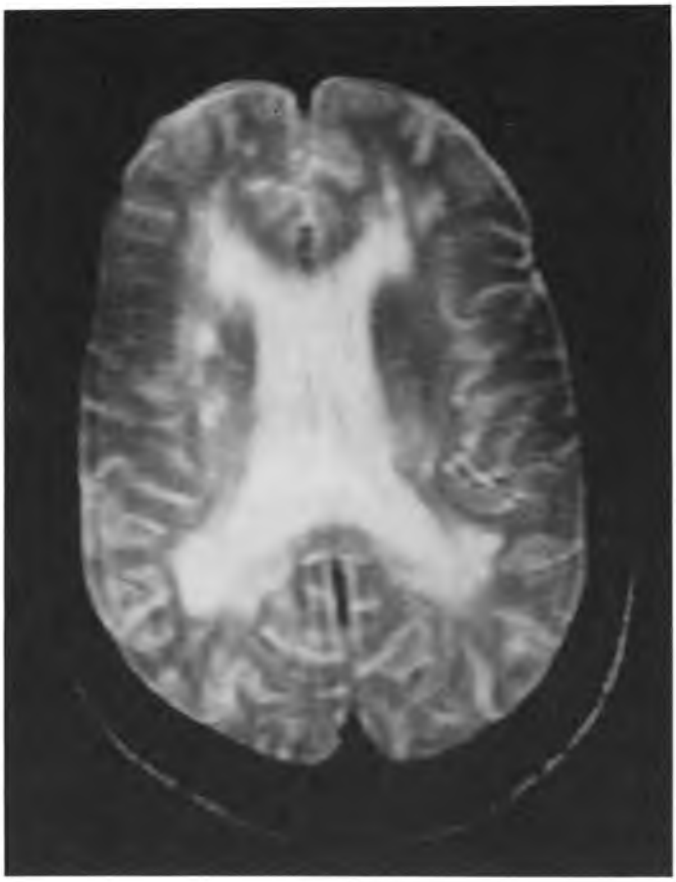


Figure 3: This transaxial T2 weighted MRI in a patient with vascular dementia demonstrates extensive confluent subcortical and periventricular white matter areas of hyperintensity.

Erkinjuntti et al. (1987) performed a comprehensive study comparing MRI and CT to see if white matter lesions differentiated vascular dementia from AD. In this study 100% of 29 patients with a clinical diagnosis of vascular dementia had white matter lesions on MRI and in 22 of 29, the lesions were rated moderate or severe. CT showed lesions in 26 of the patients but in only 9 were the changes rated moderate or severe. Conversely, in the AD population 7 of 22 patients had lesions on MRI; in 5 these changes were mild and in 2 they were moderate. Conversely, with CT 21 of 22 had no lesions while one patient had mild lesions. The authors concluded that MRI was more sensitive than CT for white matter lesions and that white matter lesions helped to differentiate between AD and multi-infarct dementia. White matter lesions were more common in patients with ischemic cerebrovascular disease, including those with vascular dementia, but white matter changes were also common in patients with AD. Similarly, in a study by Aahron-Peretz et al. (1988), 55.5% of patients with AD and 97.5% of patients with vascular dementia had white matter lesions on CT. Although both studies show that

white matter lesions are more common in multi-infarct dementia, the lesions occur in a sufficiently high percentage of normal controls and patients with AD to be used to differentiate the three conditions.

Englund, Brun and Gustafson (1989) have carried out extensive studies on the prevalence, etiology, clinical significance and pathological and biochemical characteristics of white matter lesions. In addition, they examined the relationship of these lesions to AD. Microscopically the white matter changes include "partial loss of axons, myelin sheaths and oligodendroglial cells accompanied by a fibrohyaline arteriosclerosis" (Englund et al., 1989). The changes were moderate or severe in 20%, mild in 40% and absent in 40% of all AD cases. These authors called these lesions selective incomplete white-matter infarction and hypothesized that they were due to "regionally reduced cerebral perfusion due to a subcortical small vessel disease combined with systemic hypotension."

They attempted to clinically differentiate AD patients with lesions from those without subacute ischemic white matter infarction. The group with white matter changes had more episodes of fainting and hypotension and had a stronger history of cardiac disease (84% versus 36%). The features of the dementia did not dramatically differ between the two groups of AD patients. This is the most definitive work to date on white matter disease in AD.

In summary, a large percentage of normal elderly will have at least small areas of hyperintensity visible on MRI. These changes are most commonly seen in the periventricular area on the $T^2$ weighted image and there is no evidence that the small lesions have a significant effect on cognition. The small lesions are not typically seen with CT. Approximately 10% of normal elderly will have large white matter lesions on MRI with an area of more than 2.5 $cm^2$. Lesions of this size can be seen with CT and may have subtle to severe effects on cognition. Hypertension, chronic hypotension, and ischemic cerebrovascular disease are all risk factors for white matter lesions. For this reason they are extremely common in patients with multi-infarct dementia, occurring in close to 100% of patients in two major studies. Large white matter lesions are common in patients with AD and the occurrence and progression of these lesions in AD cannot be used to rule in or rule out AD.

Ebmeier et al. (1987) quantified the changes in periventricular water by using a spin lattice relaxation time ($T^1$). This number measures the "time needed by nuclei to realign with a static magnetic field after being disturbed by two magnetic pulses in radio frequency range to $180^0$ and then to $90^0$ to the original field." Prolongation of $T^1$ can be due to an increase in free water and/or a decrease in structural elements.

In Ebmeier's study, patients with MID had a greater mean $T^1$ than did normals or patients with AD in the frontal, mid and posterior portions of the brain. The differences were most marked in the patients with periventricular changes on MRI, but $T^1$ changes overlapped between the two groups so that $T^1$ alone could not be used to differentiate patients with MID from those with AD.

Christie et al. (1988) also found that $T^1$ values were similar in AD and age-matched controls although $T^1$ values tended to increase with progression of the dementia. In this study both multi-infarct dementia and Koraskoff patients had prolonged $T^1$ values. These findings are not surprising and, like a large VB, a prolonged $T^1$ may be statistically different between groups but it does not help with clinical classification of an individual patient. Although $T^1$ relaxation time is quantitative, the exact parameters that contribute to the $T^1$ are unknown. It is not clear that T1 relaxation times offer anything more than a visual inspection of scans by experienced neuroradiologists.

Issues that need to be addressed in the future include: Why are some individuals with large white matter lesions demented while others are normal? How large do these lesions need to be before an individual becomes symptomatic and most importantly, can they be prevented?

NMR Spectroscopy and Alzheimer Disease

Nuclear magnetic resonance spectroscopy (NMR) was developed in the 1940s to determine the magnetic characteristics of atomic nuclei and has been used in molecular physics and chemistry for over 30 years. In 1976 Dawson et al. (1976) used $^{31}P$ NMR to study living muscle and shortly afterward, Chance et al. (1979) used NMR to study brain tissues. Since that time, many investigators have applied this technique to the study of metabolism and biochemistry in laboratory animals and man. Although the initial studies in laboratory animals used small magnets with strong field strengths, recent applications have utilized 1.5 Tesla magnets to study muscle, liver, heart and brain in humans.

The basis for NMR is that when an atom is subjected to an external magnetic field the nucleus will resonate at a shifted frequency which is the product of the gyromagnetic ratio and the local field strength of the magnet. The site and intensity of this shift can be measured, giving a semi-quantitative measurement and localization of a specific compound. Although most studies in humans have involved $^1H$ and $^{31}P$, in the future, it will be possible to utilize other compounds including carbon, fluorine, nitrogen and sodium.

The potential applications for NMR seem enormous. The advantage of this technology is that it allows the rapid measurement of specific compounds within small regions of the brain without disturbing the tissue environment. These measurements can be done rapidly and, with in vivo studies of muscle one can measure ATP, inorganic phosphate, phosphocreatine and pH associated with rest and with exercise. As refinements of this technique continue it will become possible to measure hundreds of molecules within the brain during rest and with brain activation. The physical theories underlying NMR spectroscopy will not be discussed here, but rather we will focus on recent applications of NMR spectroscopy to AD. Those interested in more basic information are referred to reviews by (Chance B., et al., 1979) (Radda G., 1986) (Barany M., et al. 1988).

Two types of spectroscopy, hydrogen ($^1H$) and phosphorous ($^{31}P$), are currently being used in clinical research centers to

evaluate brain chemistry. With $^{1}H$ spectroscopy there are 4 main peaks; a large peak for water, and smaller peaks for phosphocreatine, choline-containing compounds, and N-acetylaspartate. (See Figure 4). More sensitive systems are being developed that can measure lactate and glutamine. We have demonstrated that the choline peak in brain measures water-soluble compounds including choline, glycerophosphorylcholine and phosphorylcholine while it does not identify ethanolamine or ethanolamine containing compounds. Also, It does not measure membrane-bound phosphatidylcholine (unpublished data). It is possible that choline, lactate and possibly glutamine peaks will help to understand the pathogenesis and/or diagnosis of AD.

Hydrogen spectroscopy has shown specific abnormalities with certain metabolic conditions. For example, Luyten et al. (1989) showed a glutamine peak in patients with chronic hepatic encephalopathy that was not present in normals. Some authors have shown that AD is associated with a loss of large glutamutergic neurons (Bowen et al., 1987). Glutamate, GABA and glutamine are potentially resolvable peaks with $^{1}H$ spectroscopy and NMR could be used to test these biochemical findings.

$^{31}P$ spectroscopy has been extensively used to study patients with AD Barany et al.,1985) (Pettegrew et al., 1987). $^{31}P$ spectroscopy demonstrates peaks for ATP, inorganic phosphorous and phosphocreatine. In the brain there are phosphomonoester peaks that consist of a mixture of phosphorylcholine and phosphorylethanolamine and diester peaks that include glycerophosphorylcholine and glycerophosphorylethanolamine.

Figure 4.

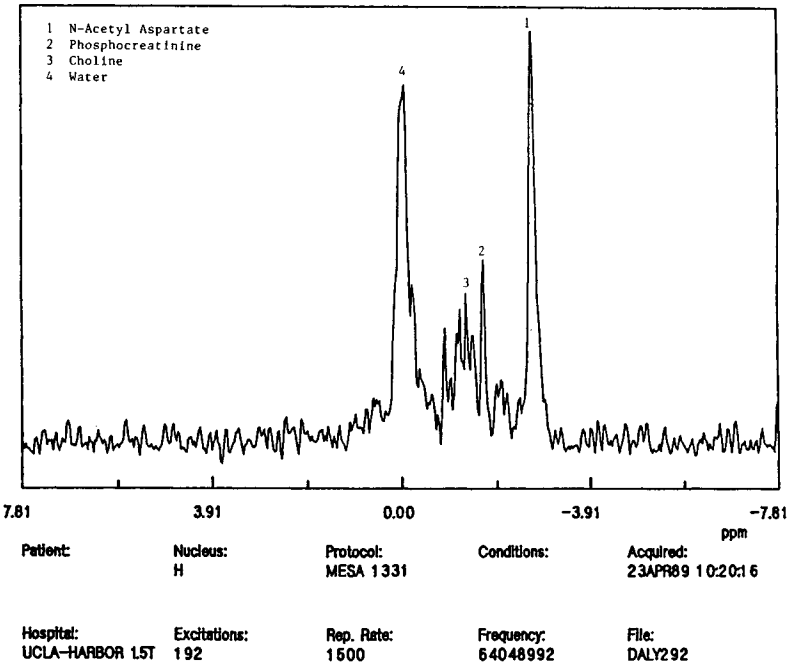


Figure 4: This is a 64 $cm^3$ NMR $^1H$ spectra from the right frontal region of a normal subject. It shows a large water peak and small peaks for choline-containing compounds, phosphocreatine/creatine and N-acetylaspartate.

Most of the work with $^{31}P$ in AD has focused on pathological changes in the brain (1986, 1987, 1988, 1989), although there have been recent in vivo studies done by Pettegrew et al. (in press). The first in vitro autopsy studies were done by Barany et al. who showed an elevation of glycerophosphorylcholine compared to glyceryolphosphorylethanolamine. Wurtman et al. (1989) with chemical methods, verified the finding of increased brain glycerophosphorylcholine in AD.

Why these phosphodiesters accumulate is unknown. However, both glycerophosphorylcholine and glycerophosphorylethanolamine are products of phospholipid degradation produced by the action of phospholipase A1 and A2 on the phosphatidyl forms of these compounds. It is possible that there is increased catabolism of brain phospholipids in AD and Farooqui et al. (1988 and 1989) have shown that the concentration of monoacyl and diacylglycerol lipases is 7 times higher in AD autopsy brain compared to non-demented elderly. These findings warrant further investigation.

Pettegrew reported extensive changes in phosphomono and phosphodiesters of choline and ethanolamine in the brains of patients with AD with in vivo NMR spectroscopy (Pettegrew In Press). Specifically, he found that in addition to glycerophosphorylcholine and glycerophosphorylethanolamine that, phosphorylcholine and phosphorylethanolamine were elevated in AD brains. Pettegrew notes that the phosphomonoesters are anabolic building blocks necessary for the production of brain phospholipids and their accumulation could mean a metabolic block. Conversely, it might mean that there was an excessive degradation of the diesters by phospholipase C although this has not been demonstrated in chemical studies (Kanfer et al., 1987).

Although some of this work is still preliminary, what is particularly compelling is that these metabolic abnormalities, including the accumulation of phosphomono and diesters, occurred in AD brain before the deposition of plaques and tangles and in regions (e.g. cerebellum) where plaques and tangles are uncommon. What spectroscopy seems to offer is the opportunity to study metabolism in vivo in the brains of normal elderly versus patients with AD.

Although most of the spectroscopy work has focused on phosphorous containing compounds, study of other chemicals may also be fruitful. Changes in brain glutamine, GABA, glutamate and lactate have already been demonstrated in laboratory animals with different disease states and examination of these compounds should be applied to the study of AD.

## Conclusions

As has been noted throughout this chapter, CT and MRI will demonstrate brain atrophy that occurs in association with AD. Unfortunately, atrophy is not specific to AD and cannot be used for the purpose of diagnosis. Both CT and MRI are useful in delineating potentially treatable vascular, neoplastic and hydrocephalic pathology that can cause a dementia syndrome.

White matter lesions are seen with CT in most patients with multi-infarct dementia and approximately one-half of patients with AD. They are less common in normal elderly. However, with MRI small lesions are common even in normal elderly. The small lesions have little clinical consequence, although larger lesions often will affect cognition. Frequently, the white matter lesions have a vascular etiology. In vivo NMR spectroscopy is in its infancy but offers great promise for better understanding the pathogenesis of AD.

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# Positron Emission Tomography and Neuropsychological Studies in Dementia

Randolph W. Parks and Robert E. Becker
*Unit on Geriatric Psychopharmacology, LCS, NIMH, Behesda, MD* and
*Southern Illinois Univ. School of Medicine, Springfield, IL*

Kathryn L. Dodrill and Bettina A. Bennett
*South Florida State Hospital, Pembroke Pines, FL*

David J. Crockett, Trevor A. Hurwitz, Patrick L. McGeer
and Edith G. McGeer
*University of British Columbia, Vancouver, BC, Canada*

## INTRODUCTION

Dramatic improvements in our ability to image the human brain in vivo have expanded researchers' skills in the early diagnosis of Alzheimer's Disease (AD). One of these techniques, Positron Emission Tomography (PET), has yielded new significant neurophysiological and cognitive data (Friedland, Horwitz & Koss, 1988; Metter, 1988; Powell & Benson, 1990) that shows a distinctive profile for assisting in a diagnosis of AD (Cutler, 1988, Friedland, 1989). This is particularly encouraging in light of the generally non-specific findings of MRI, CT, and x-ray. PET scanners recently emerged through the integration of several fields of science. Physicists and nuclear engineers developed the essential hardware to measure radioactivity. Physiologists, chemists and statisticians developed models to measure the uptake and decay of radionuclides in brain tissues. Medical doctors and neuropsychologists then developed scanning procedures, experimental design and interpretation of the obtained neurophysiological and neuropsychological data (Parks, Loewenstein & Chang, 1988; Morihisa, 1990; Parks et al. in press).

In this section, we will review PET studies of the normal brain as well as AD in relationship to neuropsychological functions. We will examine a pattern analysis of PET AD scans and demonstrate how these PET AD scans show a characteristic profile in comparison to other types of dementias.

## SENSORY SYSTEM

Generally, PET studies have focused on specific sensory pathways from the primary organs of sensation to their cortical counterparts. Observations are usually taken during conditions of sensory deprivation in order to reduce extraneous cognitive and behavioral activation on the part of the subject. Stimuli are usually presented during the resting state and may be presented to one side of the body or the other. The complexity of the stimuli is often varied in order to determine whether this aspect of the stimulus is associated with finite patterns of arousal. Finally, subjects may represent different patient groups or may be samples from a single disorder.

### SOMATOSENSORY-MOTOR STIMULATION

Greenberg et al. investigated the effects of tactile stimulation on FDG uptake. The tactile stimulus was a rapid, light stroking of the volar and dorsal surface of the fingers of the left arm with a hand-held brush which was just stiff enough to cause an appreciable stimulus without causing any discomfort. A different set of subjects were presented with stimulation to the right hand. The results supported previously obtained data gathered from subjects undergoing neurosurgical procedures: the somatosensory input caused the postcentral gyrus contralateral to the stimulus to become metabolically more active than the homologous area in the ipsilateral cortex. Alavi et al. (1981) reported greater contralateral than ipsilateral postcentral cortical activity in a similar procedure using sensory deprived subjects. These studies also reinforced the concept of high specificity of cortical representation of somatosensory pathways.

Correlational analysis has been used in order to examine patterns of activity in response to somatosensory stimulation. Clark et al. (1984) used mild electrical shocks applied to the right forearm of the subjects. They reported correlations commensurate with the contralateral and ipsilateral representation of the sensory pathways in the cortex.

Ginsberg et al. (1987 & 1988) in a series of studies used both FDG metabolism rates and blood flow by labeled water to measure contralateral increases in the somatosensory cortex. Procedures included identification of patterns presented on domino-like tiles or traced in the palm of the hand as well as the presentation of continuous vibrations to the face and fingers. Expected contralateral increases in metabolic rate were found when the procedures using touch-identification were used. No consistent significant changes were found when vibratory stimulation was presented. Additionally, these studies indicated that attention was a significant factor in activation. There was also data suggesting that there is greater left hemisphere involvement in cognitive tasks that have tactile and object recognition components.

In summary, the early studies indicated that stimulation resulted in focal increases in FDG metabolism in brain areas contralaterally located to the side of the tactile stimulation. The more recent studies produce evidence indicating that the left hemisphere is measurably more

influential in the cognitive component involved in object recognition tasks while there are more global increases in brain activity from resting state to measured activation levels. Such nonspecific changes may reflect the influence of extraneous variables including attention and arousal.

## VISUAL STIMULATION

Alavi et al. (1981) and Greenberg et al. (1981) have also studied the visual pathways. They found an increase in the uptake in the striate cortex contralateral to the stimulated visual field in normal subjects. They used a visual stimulus, moving abstract color images, or black and white lines moving at varying orientations. The subject's visual field was limited to specified hemifields. Their studies complemented existing knowledge of the visual pathway with results linking hemifield visual stimulation to increased VDG uptake in the calcarine area in all subjects.

Phelps et al. (1981) and Phelps, Kuhl & Mazziotta (1981) conducted a series of experiments in which volunteers were presented with increasingly complex stimuli varying from white light to a black and white checkerboard pattern. Stimulation with white light produced bilaterally twice as much metabolic activation in the primary visual cortex (Brodmann area 17) than did the associative visual cortex (Brodmann areas 18 and 19). The more complex stimulation of the black and white checkerboard pattern produced significantly more activation of the associative visual cortex than of the primary visual cortex although area 17 also showed increased stimulation when compared with white light stimulation.

Methological problems implicit within all of these designs make it difficult to interpret the exact relationship between the best specific stimuli components and resultant metabolic activity in the visual cortex. However, there is some suggestion that as the complexity of the visual stimulus increases, local metabolic rate activity increases more rapidly in the associative areas than in the primary areas. This change in the pattern of activation may reflect the delegation of analysis of more complex visual material to additional areas by those areas in the visual cortex responsible for primary visual analysis.

## AUDITORY STIMULATION

Alavi et al. (1981) located increased activity in the right temporal lobe regardless of which ear was stimulated, lending support to the hypothesis that the right hemisphere contributes to the processing of linguistic information. In contrast, (Greenberg et al. (1981), found evidence supporting the more traditional functional model of the auditory system based on neurophysiological studies in humans and animals: stimulation presented monaurally produces increased FDG uptake in the auditory cortex contralateral to the ear stimulated. These results were consistent with results found by Cullen et al. (1975) who also found a predominance of crossed pathways in the human auditory system.

In a series of experiments, Mazziotta et al. (1982 & 1984) have demonstrated that there are significant increases in metabolic rate in multiple brain areas after monaural presentation of tape recorded material. Areas affected include the left frontal cortex, left thalamus, bilateral posterior cortex, and the transverse temporal cortex. Specific testing conditions including variations in verbal and musical meaningfulness and sophistication of the subjects influenced the results significantly.

The fact that these thematically similar studies have produced somewhat contradictory results indicates that future studies in this area must be much better controlled. At least one aspect that deserves further study is the effect of the complexity of the stimulus presented to the subject. Not only must the sensory complexity be controlled, but the meaning or significance of the stimulus must be kept constant. The temporal spacing or density of the stimulus is another aspect of the experimental design that has yet to be adequately investigated. Information provided in a paced manner may allow the identification of compartments of activation that reflect the different roles of sensory processing.

## RESTING STATE PET AND COGNITIVE FUNCTIONING

PET scan studies measuring cognitive activity during resting states also utilize conditions that minimize sensory and motor input. The subject is supine in a room with eyes covered and ears plugged during the scanning process. Neuropsychological testing is conducted either before or after the scanning process. Cognitive test scores are then correlated with the resting state metabolic values produced with scanning. Because levels of metabolic activities are taken during resting states and therefore the subject's cognitive activity is not controlled, metabolic values do not accurately reflect specific cortical activity during cognitive tasks. Moreover, the fact that neuropsychological test scores are obtained at a different time than the PET values, it is difficult to relate level of performance on these tasks to specific patterns of metabolic activity.

This type of procedure is especially valuable with subjects who suffer from cognitive impairments such as seen in AD or others for whom test materials presented during scanning would produce unwanted activation during the scanning process (Benson, 1982; Duara et al., 1986; Foster et al., 1983; Friedland et al., 1983, Haxby, 1984; Huff, 1984; McGeer et al., 1986). There are significant problems associated with data obtained using this correlation procedure since the two measurements are obtained separately. Additionally, there may be significant variation in attention and cognitive functioning during resting state measurement resulting in an inaccurate magnitude of difference in FDG uptake between subjects.

A number of studies on normal subjects who have been screened for health have consistently revealed no correlations between indices of cerebral metabolism and performance on neuropsychological tests (Duara et al., 1984; Haxby et al., 1986). However, when the subjects were

seriously cognitively impaired, the rate of FDG uptake in the frontal cortex, the temporal cortex, and the caudate nucleus correlated significantly with the degree of cognitive impairment (Farkas et al., 1982).

Friedland et al. (1983) and Chase et al. (1983, 1984, 1985) variously correlated subtests of the Wechsler Adult Intelligence Scale, the Mattis Dementia Rating Scale, or the Wechsler Memory Scale with glucose metabolism in the right hemisphere, the left hemisphere, and/or the whole brain. Results included a correlation between performance IQ scores and left but not right hemisphere functioning; whole brain metabolism correlated negatively with Alzheimer's diagnosis; metabolism in both hemispheres correlated with overall intellectual functioning; verbal subtest scores correlated with cortical metabolism in the left hemisphere while nonverbal test scores correlated with metabolism in the right hemisphere.

Local cerebral metabolic rates for glucose in 32 regions of interest were reported upon my McGeer et al. (in press) on patients with clinically diagnosed AD and 20 neurological normal controls. This study also reported serial investigations on 13 AD patients. At the same time, extensive neuropsychological testing was done on all AD patients. In almost all cortical regions, there was a significant decline in local cerebral metabolic rates for glucose with age in the control subjects. There were the expected cortical metabolic deficits seen in AD. Moreover, the serial study showed a general increase in such deficits over time in 12 of the 13 cases. The region showing the greatest decline with time in the serial study is the same as showing the most severe deficiencies in cross sectional studies. Results on individual neuropsychological tests tend to correlate with metabolic rates in multiple rather than single, cortical regions, suggesting intact neural networks are required for good performance. The correlation with cortical metabolic activity indicated that the higher the metabolic rates, the better the performance. In addition, a better balance in activity between the right and left hemispheres as reflected by left right asymmetry index was also associated with better performance on the neuropsychological test.

## COGNITIVE "CHALLENGE" STUDIES DURING PET SCANNING

The major problem with resting state studies is that there is no opportunity to observe simultaneous brain behavior relationships. Recent advances in testing procedures allow the examiner to obtain two separate PET scans during a single testing session (Gur et al., 1983) or to perform statistical corrections that account for covariance (Cronbach & Furby, 1970; Weisberg, 1979; Bartlett et al., 1987). At this point, it should be noted that much of the PET scan data does not meet the assumptions necessary to allow the data to be analyzed using regression analysis. In these cases, it might be much more appropriate to use Multiple Classification Analysis (Andrews, 1973). This type of analysis is associated with much less stringent set of assumptions regarding the nature of the data and still allows for the examination of general differences in patterns of analysis.

## PET RESEARCH OF OTHER DEMENTIAS

### Cruetzfeldt-Jakob Disease

Cruetzfeldt-Jakob (CJD) Disease patients typically exhibit cognitive deficits and gait difficulties that are initiated by a virus. In a case study of a middle aged man (Friedland, Prusiner, Jagust, Budinger & Davis, 1984), the CJ patient showed an asymmetric temporal metabolic abnormality that was not similar to parietal and temporal deficiencies in metabolism when compared to an AD group. This asymmetry in temporal metabolism was also evident when compared to a group of normal subjects.

### Huntington's Disease

Huntington's Disease (HD) is characterized by the prominence of spasmodic, involuntary movements, cognitive and personality disturbance depending upon onset (Caine, Hunt, Weingartner & Ebert, 1978; Paulson, 1979). Resting state PET scans of at risk HD patients have not yielded consistent findings between research institutions. There seems to be a general consensus that learning and memory deficits are related to the course of HD learning and memory deficits are related to the course of HD (Berent et al., 1988; Butters, Granholm, Salmon & Grant, 1987) and that these changes in the metabolism of the caudate have been demonstrated in subjects with HD (Berent et al., 1988; Kuhl et al., 1982; Hayden, Martin & Stoessl, 1986) and those at risk for HD (Mazziotta et al., 1987). Using different techniques to analyze their PET scans, Young and associates (1987) did not find lowered metabolism in the caudate of persons at risk for HD. These mixed findings perhaps may partially be accounted for by different age groups of subjects, selection criteria for assessment of HD and models for interpretation of PET scan data.

Recent advances in human medical genetics have enabled researchers to identify a DNA polymorphic marker located on the distal region of the short arm of chromosome 4 (Gusella et al., 1983). Within limitations, investigators with facilities for gene-map assignment of the crucial area of chromosome 4 (D4S10) are able to divide those at risk for HD into two groups, those who might not have inherited the mutant gene and another cohort who are presymptomatic heterozygotes for HD (Wang, Greenberg, Hewitt, Kalousek & Hayden, 1986; Hayden et al., 1987). In a PET resting state glucose study in which DNA results were available for some subjects, Hayden et al. (1987) did find that caudate metabolism was abnormally low in some presymptomatic persons at risk for HD who were likely to have inherited the mutant gene, however, the PET findings were not clearly abnormal in all of HD+ subjects. Because these changes were noted in only some of the HD+ subjects (i.e. 3 out of the 8), still at issue is whether these metabolic changes in the caudate are clinically useful for discriminating between subjects at high risk (HD+) of developing HD from those subjects with a lower probability of HD (HD-).

### Multiple Infarct Dementia

Multiple Infarct Dementia (MID) is a vascular disease that usually shows a progressive stepwise course. The nature of the cognitive

impairment is associated with the severity of focal lesions. Diffuse cognitive deficits are noted with the increased prevalence of infarction (Roth, 1978; Lezak, 1983). Kuhl and associates (1985) have shown how MID differs from AD and normal subjects using FDG resting state PET scans. MID patients demonstrated scattered local metabolic defects in the cortex, caudate, thalamus, white matter, and cerebellum. CT scans of the same MID patients showed fewer structural lesions that were smaller in extent, however, FDG scans missed lesions of small lacunar infarcts and abnormal white matter. Since regional metabolic ratios for AD patients were lowest in the parietal cortex and highest in caudate-thalamus, a ratio of these metabolic values were able to separate AD patients from normal subjects and MID patients. In general, Kuhl and colleagues (1985) found that the typical PET MID scan consisted of random focal deficits in an otherwise normal metabolic distribution and that PET scans were more sensitive to the presence of stroke than CT scans.

### Normal Pressure Hydrocephalus

Normal Pressure Hydrocephalus (NPH) is a reversible condition which usually results from obstruction of cerebral spinal fluid flow. These patients usually show incontinence, gait disturbance, memory impairment and other forms of cognitive dysfunction. When compared to normal and AD subjects, NPH show lower overall glucose metabolism (Jagust, Friedland & Budinger, 1985). In the same study, it was shown that NPH patients did not show focal deficits in metabolism, whereas the AD subjects demonstrated temporal and parietal hypometabolism.

### Pick's Disease

In an advanced stage of Pick's Disease (PD), patients show impaired memory, disorientation, and other cognitive deficits. Histopathology shows grossly swollen neurons that contain classic "Pick" bodies as well as circumscribed frontal atrophy (McGeachie, Fleming & Sharer, 1979). In a case study of PD confirmed by necropsy, PET scan confirmed reduced frontal metabolism when compared to normal controls (Kamo et al., 1987). Furthermore, the PD case was distinct from AD cases in showing a much greater decrease of regional metabolism in the frontal than in the temporal or parietal lobes.

## ALZHEIMER'S DISEASE

### PET Neurophysiological Tracer Kinetics

PET tracer kinetics of radiopharmaceuticals are playing an increasing role in probing the basic mechanisms underlying AD PET hypometabolism. By using a modified Sokoloff et al. (1977) three compartmental model to describe the transport of FDG in AD and healthy controls, Friedland and coworkers (1989) suggested that glucose transport from the blood to the blood-brain barrier ($K^1$) and reverse transport processes ($K^2$) are normal in AD. However, phosphorylation of hexokinase ($k^3$) was reduced in the temporal lobe of AD patients, suggesting "deficient glucose utilization in AD is due to decreased activity at the hexokinase step . . ." (Friedland et al., 1989, p. 1431). Marcus and

colleagues (1989) also found that microvessels from the temporal cortex in AD showed decreased glucose uptake when compared to controls. This was related to decreased hexokinase activity ($k^3$), suggesting AD patients are impaired with respect to using glucose as an energy source.

Pattern Analysis of AD PET and Conclusions

AD PET scans usually show a decline in overall glucose uptake when compared to age-matched normal controls but not in MID and depression (Kuhl, Metter & Riege, 1985). Regional differences in AD metabolism are also salient in the parietal, temporal and frontal lobes (Farkas et al., 1982; McGeer et al., 1986). Parietal ratios of FDG uptake have been particularly useful in separating AD from normal adults (Duara et al., 1986) and the sensitivity of parietal metabolic ratios in AD have been independent of gender effect (Small et al., 1989).

Studies have shown that severely demented subjects show larger decrements in FDG uptake than do mildly demented AD patients (McGeer, 1986; Jamieson, Chawluk & Alavi, 1987). In asymmetric (Haxby et al., 1986; Rapoport; 1986) and these asymmetries in FDG correlate with decline in neuropsychological function (Foster et al., 1984; Grady Haxby, Schlageter, Berg & Rapoport, 1986; Haxby, Duara, Grady, Cutler & Rapoport, 1985). Metabolic asymmetries appear to be relatively stable over time (Grady et al., 1988; Haxby, Grady & Koss, 1988), but not necessarily stable over time in all brain regions (Jagust et al., 1988; McGeer et al., in press). The problematic nature of multiple correlations found between results of many psychological tests and resting state regional FDG uptake suggests several hypothesis (McGeer et al., in press): (1) performance on a given test may depend primarily on one cortical region but multiple correlations appear significant because the metabolic deficiency is increasing in a large number of regions at very similar rates; (2) each neuropsychological task involves a network of cortical regions (Parks et al., 1988), and metabolic deficiency in any one can contribute to defective performance task (Parks, Crockett, Tuokko et al., 1989; Parks, Crockett & McGeer, 1989); and/or (3) the primary and early problem in AD is loss of hippocampal neurons serving short-term memory and the effect of progressive deterioration in this region, not studied in most PET work, on neuropsychological test performance outweighs any concurrent cortical degenerative processes that may be occurring in the disease. Since most neuropsychological tests are multi-factoral in sensory performance, the second and third hypotheses may be of more importance.

The practical clinical importance of PET has been clearly demonstrated with this technology. Even visual interpretation of PET scans are more than 90% accurate in separating AD patients from healthy normal subjects (Alavi, 1989). Finally, performance of a neuropsychological test during PET scanning may increase the diagnostic sensitivity of imaging procedures in the detection of AD (Miller et al., 1987).

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# Visualization of Cholinergic Tracers in Alzheimer Brains in vivo by Positron Emission Tomography

Agneta Nordberg,[1,6] Per Hartvig,[5] Hans Lundqvist,[3] Andres Lilja,[4] Matti Viitanen,[6] Kaarina Amberla,[6] J. Ulin,[2] Bengt Winbald,[6] and Bengt Långström[2]

[1] *Departments of Pharmacology,* [2] *Organic Chemistry,* [3] *The Svedbergh Laboratory,* [4] *Diagnostic Radiology, University of Uppsala* [5] *Hospital Pharmacy, University Hospital, Uppsala* and [6] *Department of Geriatric Medicine, Huddinge Hospital, Huddinge, Sweden*

## INTRODUCTION

Information about the cholinergic system in the human brain has mainly been obtained from neurochemical studies performed in autopsy but rarely also in biopsy brain tissue. The neurochemical analysis have mainly been restricted to measurement of enzyme activities (acetylcholinesterase, choline acetyltransferase) (Perry et al. 1986) and cholinergic muscarinic (Nordberg and Winblad 1981, 1986a) and nicotinic receptor densities (Nordberg and Winblad 1986b). The neurochemical postmortem brain studies that have been performed in Alzheimer patients indicate deficiencies in several transmitter systems (for review see Hardy et al. 1985, Gottfries 1985). Recently, new techniques using radionucleotides have made it possible to measure in autopsy brain tissue dynamic, functional mechanisms such as transmitter release (for review see Dodd et al. 1988). The most consistent neurochemical changes in brain that have been observed are related to the cholinergic system. This transmitter system correlates best with cognitive function in animal and man (Drachman 1977, 1978).

There is still no complete agreement between clinical diagnosis and final histopathological examination of Alzheimer patients, which highlights the need for an improved diagnostic resolution. Positron emission tomography (PET) is a technique for in vivo studies of central neuronal activity in brain. The method has already shown a great potential for psychopharmacological research (Phelps et al 1982). The PET technique is therefore of interest as a putative diagnostic tool in neurodegenerative diseases such as Alzheimer's disease. Possibly, abnormalities visualized by PET will preceed changes revealed by CT and make early therapeutic interventions possible. The PET studies that have been performed in Alzheimer patients are limited and mainly concern cerebral blood flow and metabolism (oxygen utilization, glucose utilization) (Frackowiak et al 1981, Ferris et al 1983, Riege and Metter 1988, Burns et al 1989). A decreased cerebral blood flow, oxygen utilization and glucose utilization have been observed especially in the temporal, parietal but also in the

frontal cortical areas of Alzheimer brains.

PET is a technique suitable for in vivo studies of central cholinergic activity in brain. The aim of this chapter is to focus on attempts to measure cholinergic markers (acetylcholine synthesis, muscarinic and nicotinic receptors) in brain by the PET technique with special reference to Alzheimer's disease.

## THE USE OF $^{11}$C-CHOLINE AS A MARKER FOR CHOLINERGIC ACTIVITY IN BRAIN

Choline (Ch) is the precursor for acetylcholine (ACh). Radiolabelled Ch has been injected into animals and used to measure the turnover of ACh in the brain (Nordberg 1977). The Ch high affinity uptake in brain is one of the rate limiting steps in the synthesis of ACh and can therefore be used as a marker for brain cholinergic function. In vivo PET studies using $^{11}$C-Ch have been performed both in monkey (Eckernäs et al. 1985, 1986) and man (Gauthier et al. 1985) (Table I). Recently the uptake of a choline analog $^{11}$C-pyrrolidinocholine to dog brain has been studied by positron emission tomography. The use of $^{11}$C-Ch as a radiolabelled marker for cholinergic activity in vivo in brain has however some disadvantages. Firstly, a very small proportion of the $^{11}$C-Ch is taken up by the brain following an intravenous injection. Secondly, Ch is metabolized not only to ACh but also to phosphorylcholine and betaine which limit the possibility to solely study ACh synthesis in vivo.

An alternative way to precursor transport and transmitter turnover would be to visualize the cholinergic receptors (especially the presynaptic receptors) in brain by PET technique.

## MUSCARINIC RECEPTORS IN ALZHEIMER BRAINS AS VISUALIZED BY PET

The muscarinic receptors represent together with the nicotinic receptors the cholinergic receptor systems in the brain. Modern molecular biology techniques have recently revealed a whole family of muscarinic receptor genes coding for the muscarinic receptors in the brain (Bonner et al. 1987). Pharmacological agonists/antagonists to characterize all these receptor subtypes is still lacking. In Alzheimer's disease the muscarinic receptors have mainly been preserved when analysed in postmortem brain tissue (Nordberg and Winblad 1986a). The muscarinic antagonists $^{11}$C-benztropine mesylat and $^{11}$C-3-quinuclidinylbenzilate ($^{11}$C-QNB) have been used in attempts to visualize muscarinic receptors in vivo in baboon brains (Dewey et al. 1989, Mazière et al. 1989). Hollman et al. (1985) used the muscarinic antagonist $^{123}$I-quinuclidinyl-4-iodobenzilate and single photon emission tomography (SPECT) to visualize muscarinic receptors in human brain. In this very limited study of one control and one Alzheimer patient the binding of $^{123}$I-quinuclidinyl-4-iodo benzilate was uniformly and only moderately decreased in brain of the Alzheimer patient compared to the control subject.

## NICOTINIC RECEPTORS IN BRAIN AS VISUALIZED BY $^{11}$C-NICOTINE

During recent years the presence of heterogenous nicotinic receptors in brain has been characterized by in vitro receptor

Table I

Brain imaging studies to visualize cholinergic tracers in human brain.

| | |
|---|---|
| **PET** | |
| *Choline uptake, ACh synthesis* | |
| $^{11}$C-Choline | Gauthier et al. 1985<br>Eckernäs et al. 1985 |
| *Nicotinic receptors* | |
| (-) and (+) $^{11}$C-nicotine | Nybäck et al. 1989<br>Nordberg et al. 1989a |
| **SPECT** | |
| *Muscarinic receptors* | |
| $^{123}$I-quinuclidinyl-iodo-benzilate | Hollman et al. 1985 |

binding studies in rodent and human brain tissue (Nordberg et al. 1988b). The assumption of multiple brain nicotinic receptor subtypes is supported by molecular biology techniques (Patrick et al. 1989). Attempts to visualize nicotinic receptors in vivo using $^{11}$C-nicotine and PET have been made in monkey (Mazière, 1976, Nordberg et al. 1989b) and man (Nybäck et al. 1989, Nordberg et al. 1989a). (-) $^{11}$C-nicotine given as a bolus dose is rapidly taken up and distributed to various parts of the monkey and human brain. The $^{11}$C-radioactivity peaks within 5 mins followed by a decrease during 15-20 min (Nordberg et al. 1989b, Nybäck et al. 1989). The regional distribution of $^{11}$C-nicotine in brain revealed by PET is very similar with the in vitro mapping of nicotinic receptors (Larsson and Nordberg 1985, Härfstrand et al. 1988, Adem et al. 1989). Thus the uptake of $^{11}$C-nicotine is high in areas as the thalamus, caudate nucleus, putamen, frontal and temporal cortex, intermediate in the occipital cortex, cerebellum and low in the white matter. Due to the small size of the monkey brain the anatomical resolution in that specie using PET is less precise compared to human brain. The (+) and (-) enantiomers of $^{11}$C-nicotine show a similar regional distribution in monkey and human brain. When the peripheral nicotinic receptors in the monkey was blocked by trimetaphan infusion the uptake of (+) $^{11}$C-nicotine to the brain decreased and the differences in regional distribution was lost while the uptake and the regional distribution of the (-) enantiomer was retained (Nordberg et al. 1989b). It is speculated that the difference in uptake between the (+) and (-) isomers under peripheral nicotinic receptor blockade represents the high affinity nicotinic binding of (-) $^{11}$C-nicotine (Nordberg et al 1989a).

Higher uptake of (+) and especially (-) $^{11}$C-nicotine to brain has been observed in young smokers compared age-matched

non-smokers (Nybäck et al. 1989). The observation agrees with findings of an enhanched number of high affinity nicotinic receptors in postmortem brains of smokers (Benwell et al. 1988) and nicotinic receptor increase in animals following nicotine treatment (Romanelli et al. 1988).

## UPTAKE OF $^{11}$C-NICOTINE IN ALZHEIMER BRAINS

Neurochemical studies in postmortem brain tissue from Alzheimer patients have revealed a marked deficit of the cortical nicotinic receptors (Nordberg and Winblad 1986b, Whitehouse et al. 1986). Furthermore a change in the proportion of high affinity to low affinity nicotinic binding sites has been observed (Nordberg et al. 1988a). The decrease in nicotinic receptors is similar to the decrease in choline acetyltransferase activity and ACh release and might be a marker for presynaptic defects (Nordberg and Winblad 1986, Nordberg et al. 1989c). In order to try to visualize the nicotinic receptors in vivo the (+) and (-) isomers of $^{11}$C-nicotine have been given intravenously in a tracer dose to Alzheimer patients and age-matched controls. The clinical diagnosis of Alzheimer disease was determined according to Diagnosis and Statistical Manual of Mental Disorders DSM III-R criteria. Clinical examination included blood and cerebrospinal fluid (CSF) laboratory, electroencephalography (EEG), computed tomography (CT) and magnetic resonance imaging (MRI). Three age-matched (66±3 ys) healthy volunteers with no history of psychiatric or neurological disease served as controls. They were clinical examined and no finding was observed on EEG. A thorough neuropsychological investigation was done to all the patients and the controls. Methods such as Luria's neuropsychological methods, an applied form for investigation of demented patients, subtests from WAIS-R (Information, Similarities, Comprehension, Block design), Trail Making Test, WMS Logical Memory immediate recall and delayed recall, wordlists with immediate recall, cued recall and recognition were used (Erkinjuntti et al 1986, Reitan 1958, Wechsler 1945, 1981, Weingartner et al. 1983). The Mini Mental State Examination (MMSE) score (Folstein et al 1975) for the

TABLE II DESCRIPTION OF ALZHEIMER CASES

| | | Age years | Duration of disease (ys) | MMSE | CT and/or MRT changes sulci | ventricles |
|---|---|---|---|---|---|---|
| AD | 1 | 63 | 10 | 18/30 | + | + |
| | 2 | 65 | 6 | 9/30 | ++ | + |
| | 3 | 68 | 1 | 27/30 | + | + |
| | 4 | 71 | 5 | 19/30 | - | + |
| | 5 | 64 | 7 | 11/30 | + | + |

Alzheimer group varied due to the duration of the disease and the mean value was 17±3 out of 30 (Table II).

In the Alzheimer group a lower uptake of both isomers especially the (+) $^{11}$C-nicotine was observed in cortical brain areas such as temporal cortex, but also in the frontal cortex, compared to age-matched controls. The most striking finding was the lower uptake of (+) $^{11}$C-nicotine compared to (-) $^{11}$C-nicotine in the Alzheimer brains. This difference between the enantiomers was less pronounced in the control group. The significant lower uptake of (+) $^{11}$C-nicotine that was observed in the temporal cortex of Alzheimer patients compared to healthy non-smoking subjects is illustrated in Figure 1.

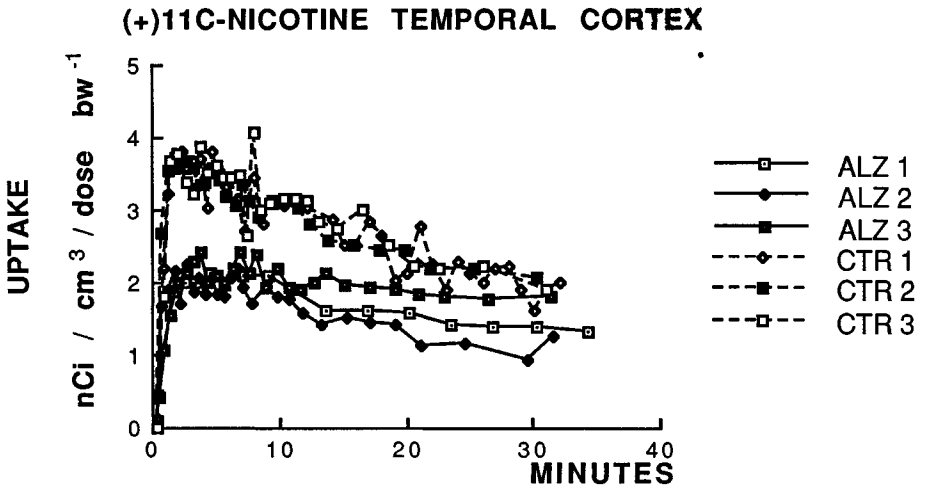



Figure 1
Uptake and time course of $^{11}$C-radioactivity in the temporal cortex following intravenous injection of (+) $^{11}$C-nicotine to Alzheimer patients and aged-matched healthy volunteers.

The patients also underwent PET studies using $^{11}$C-butanol as a marker for cerebral blood flow (Herscovitch et al. 1987). The $^{11}$C-butanol peaked within 1-2 min following intravenous injection in the brain and was more quickly eliminated from the brain in comparison to both (+) and (-) $^{11}$C-nicotine. The uptake of $^{11}$C-butanol in an Alzheimer patient is illustrated in Figure 2 where a markedly lower uptake of $^{11}$C-butanol was observed in the cortical areas compared to the thalamus. The time course for the elimination of $^{11}$C-butanol was also less steep in the frontal, temporal cortex compared to the thalamus. No significant difference in the regional distribution of $^{11}$C-butanol was observed in the control subjects. Since the time courses for both (+) and (-) $^{11}$C-nicotine differ from $^{11}$C-butanol in both control and Alzhei-

mer brains (Figure 3) it might indicate that $^{11}$C-nicotine at least partly has a specific binding profile. The finding of a lower uptake of especially (+) $^{11}$C-nicotine in cortical areas in Alzheimer brains may also indicate changes in nicotinic receptor properties (Figure 1, 3). A shift in the proportion of high affinity nicotinic binding sites to low affinity sites with a concomitant decrease in affinity has recently been observed in postmortem brain tissue from Alzheimer patients (Nordberg et al. 1988).

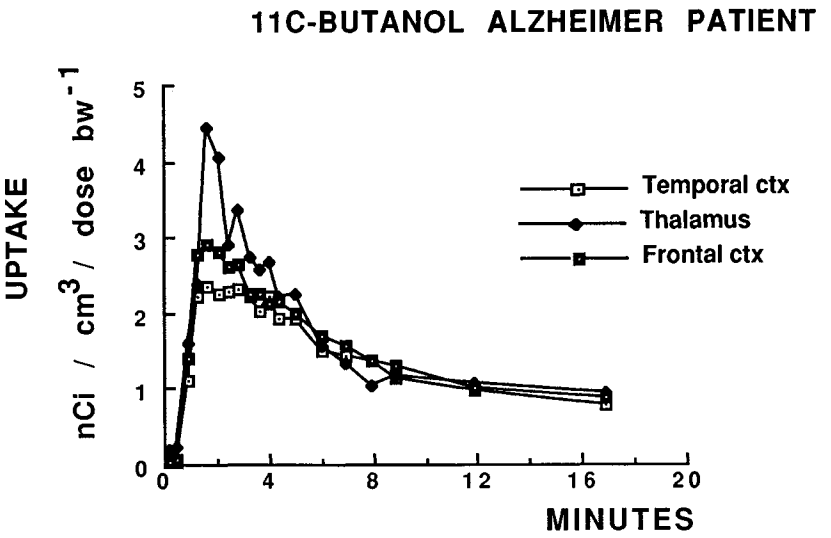



Figure 2

Uptake and time course of $^{11}$C-butanol in the temporal, frontal cortices and the thalamus of an Alzheimer patient as studied by PET.

FUTURE PROSPECTS

By using PET it is now possible to visualize receptor binding in human brain in vivo. For the cholinergic system in brain receptor studies using PET might be one way to visualize tracers for cholinergic function in brain. Since the cholinergic system is severely affected in neurodegenerative diseases such as Alzheimer's disease attempts to measure cholinergic receptor changes, especially nicotinic receptors, by PET must be promising. The nicotinic receptors in brain is already known from postmortem studies to be markedly changed in Alzheimer's disease. Since the time courses for the $^{11}$C-nicotine enantiomers are different from that of the flow marker $^{11}$C-butanol the changes in

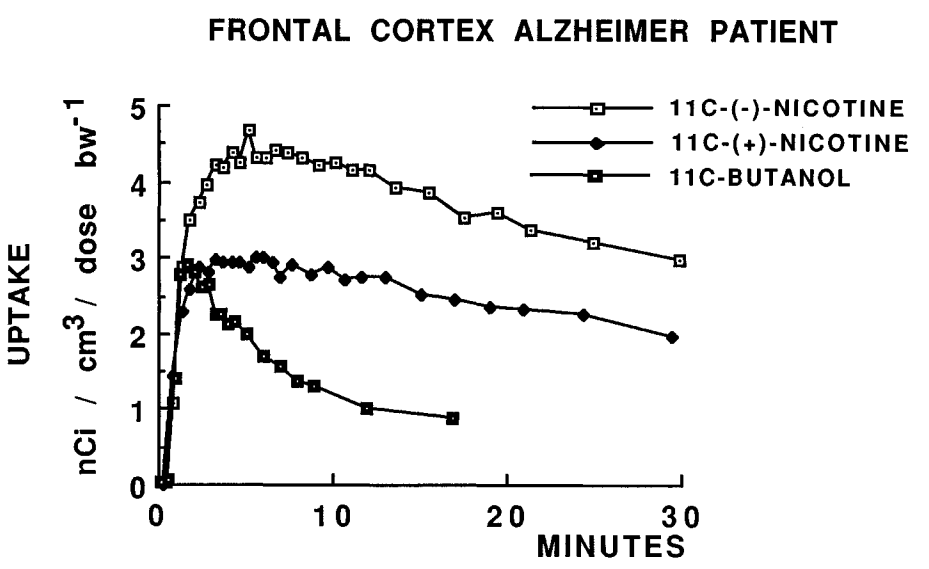



Figure 3

Uptake and time course of $^{11}$C-radioactivity in the frontal cortex following intravenous injection of (+) $^{11}$C-nicotine, (-) $^{11}$C-nicotine and $^{11}$C-butanol to an Alzheimer patient.

uptake and time course of $^{11}$C-nicotine in Alzheimer patients might reflect changes in specific binding in the brain. A further important question is how early in the course of the Alzheimer disease changes in uptake of $^{11}$C-nicotine can be detected. Presumably an early detection of the disease will favour the attempts to therapeutic intervention. Diagnosis of the different forms of Alzheimer's disease with probably different age of onset is another issue to be considered using PET and $^{11}$C-nicotine.

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish Medical Research Council, Swedish Tobacco Company, Loo and Hans Osterman's foundation, Stohne's foundation.

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# SPECT Studies in Alzheimer Type Dementia Patients

Ismael Mena, Javier Villanueva-Meyer and Jacques Darcourt
*Div. of Nuclear Medicine, Harbor-UCLA Medical Center, Torrance, CA* and
*Div.of Biophysics and Nuclear Medicine, University of Nice, France*

Neuroimaging of dementia of the Alzheimer type (DAT) has progressed rapidly during the last 7 years. Contributions of noninvasive structural imaging modalities are outlined in other chapters of this book. Contributions of positron emission tomography (PET) originates in the early observation of Kuhl et al that demonstrated that in Alzheimer type dementia there is a bilateral temporoparietal depressed utilization of [$^{18}$F]fluorodeoxyglucose (FDG) (Kuhl et al, 1983). This pattern has been confirmed by several investigators and has been observed also with rCBF measurement of cortical perfusion by the single-photon emission computed tomography (SPECT) technique. SPECT offers the possibility of incorporation of the PET findings to many university centers lacking PET and to community hospital level.

PET labelled compounds are produced in-house by means of a cyclotron and a team of radiochemists and physicists. Single photon emitter radionuclides are available in kit form and therefore 7 days a week and on a 24-hour basis. $^{133}$Xe has a physical half-life of 5.2 days and is easily obtained. It provides quantitative rCBF measurements that are complemented by high resolution studies with $^{99m}$Tc labelled radiopharmaceuticals that concentrate in the cerebral cortex, basal ganglia and cerebellum as a function primarily of blood flow. The combination of both $^{133}$Xe quantitative rCBF measurement with the high resolution $^{99m}$Tc is a powerful and comprehensive approach to the assessment of cerebral circulation in dementia of the Alzheimer type.

The availability of an array of cerebral blood flow agents for the evaluation of Alzheimer type dementia patients is complemented by several technical developments leading to better quantitative and qualitative studies.

In this chapter, we discuss the following:

1. Major radiopharmaceuticals for the evaluation of cerebral perfusion.
2. Instrumentation developments, imaging techniques.
3. Clinical applications, early diagnosis of Alzheimer's disease and other dementias. Quantitative analysis and clinical correlations.

**RADIOPHARMACEUTICALS**

Absolute quantitation of regional cerebral blood flow (rCBF) can be performed with the $^{133}Xe$ washout technique. $^{133}Xe$ SPECT studies are of poor resolution and should therefore be complemented with $^{99m}Tc$ or $^{123}I$ labelled brain imaging tracers that allow high resolution SPECT imaging.

Recently several radiopharmaceuticals that cross the blood-brain-barrier (BBB) and are retained by the neural tissue have been developed, namely [$^{99m}Tc$]HMPAO, [$^{99m}Tc$]ECD and [$^{123}I$]IMP. They enter into the brain in relation to three factors (Rapoport et al., 1979) 1) lipophilicity: passage across the BBB is by transepithelial diffusion in contrast to carrier mediated transport as is the case with glucose; 2) degree of ionization: less ionization favors brain entry, a ph gradient exists between blood (pH=7.4) and brain cells (pH=7.0), thus if the compound is lipid soluble at high pH but polar at a lower pH it will be trapped in the brain; 3) plasma protein binding: higher binding will hinder brain entrance. Once in the brain these tracers, ([$^{99m}Tc$]HMPAO, [$^{99m}Tc$]ECD, [$^{123}I$]IMP), have an adequate residence time permitting SPECT scanning.

**$^{133}Xe$**

Regional cerebral blood flow (rCBF) measurements can be accomplished based on the Fick principle that states that a substance is transported to a tissue by arterial blood less the amount carried away by venous blood. The following assumptions are made: - the tracer is metabolically inert, it does not interact with the tissue, - is freely diffusible, will cross readily the BBB, - arterial flow equals venous flow so that the clearance rate from the tissue is in proportion to blood flow. Kety and Schmidt described this relationship in 1945 using nitrous oxide (Kety and Schmidt, 1945; Kety, 1951). $^{133}Xe$ is a freely diffusible, inert, lipophilic radioactive gas, emits 81 KeV radiation and has a physical half-life of 5.25 days. Xenon is neither utilized nor produced by the body. For the calculation of rCBF $^{133}Xe$ was used initially by intra-arterial injection in the carotid artery (Hoedt-Rasmussen et al., 1966). Similar results can be achieved by using the non-invasive route: inhalation as a gas, or by intravenous injection of a saline solution were $^{133}Xe$ is dissolved. Inhaled $^{133}Xe$ passes through the alveolar wall and enters the pulmonary venous circulation. In the brain, it diffuses through the BBB and distributes in the extracellular space, exchanging freely with the blood compartment. Thus, the washout of $^{133}Xe$ distributed in the brain extracellular space and its exponential constant are a function of cerebral blood flow. As the input of $^{133}Xe$ into the brain with either inhalation or IV injection is not instantaneous, in contrast to intracarotid injection, high concentrations of $^{133}Xe$ are observed in the airways: nasopharynx and mouth. Scatter radiation from these sources distort the washout curves but this inconvenience is obviated by delaying the analysis until the $^{133}Xe$ concentration in the exhaled air reaches 20 % of the maximum, the expired air and the arterial isotope concentration curves are very similar (Obrist et al., 1975; Obrist et al., 1967). The great majority of $^{133}Xe$ is exhaled within 5 minutes. rCBF is relatively insensitive to changes in arterial oxygen concentration over the physiologic range and to acidosis or alkalosis, but rCBF is extremely sensitive to carbon dioxide concentration. The cerebral

circulatory response is approximately 5% change in rCBF for each 1 mm Hg change in arterial carbon dioxide (Harper and Glass, 1965). Therefore the need to monitor exhaled pCO2, hypocapnia decreases rCBF and hypercapnia increases rCBF. If the pCO2 value is in the range of 33-40 %/mmHg rCBF calculations are accurate, algorithms used to correct rCBF if the pCO2 is outside this range have not proved to be of value. A single or dual compartment model can be used to analyze the washout curves, depending on the equipment used, and the results are expressed in ml/100gr/min (Kety and Schmidt, 1945; Obrist et al., 1967; Kanno et al., 1979; Celsis et al., 1981; Kohlmeyer, 1985). Normal values for grey matter fluctuate between 60 - 80 ml/100gr/min.

Dosimetry - in an inhalation study the concentration of $^{133}Xe$ in the lung is typically 10 mCi/liter which is maintained for 1.5 min, the target organ is the lung which receives 0.4-0.6 rad, the gonadal dose being 0.06 rad (Atkins et al., 1980). This relatively low radiation doses permits 8 sequential or consecutive studies to be performed, while maintaining acceptable dosimetry.

## [$^{99m}$Tc]HMPAO

99m-Technetium d,l, hexamethylpropilene amine oxime (HMPAO, exametazime, Ceretec™, Amersham), is a cerebral perfusion imaging tracer (Sharp et al., 1986; Neirinckx et al., 1987). It is a lipophilic molecule that crosses the blood brain barrier in its d,l, optical configuration and has a high first pass extraction. 3.5 - 7 % of the injected dose is taken up by the brain, equilibrium is reached within 2 minutes. Only 15 % of the amount taken up by the brain may diffuse back (Anderson et al., 1988). After this period the brain concentration does not change significantly for 24 hours (De Jong et al, 1989). Once in the brain, [$^{99m}$Tc]HMPAO is converted to a meso-optical hydrophilic form, that is trapped and cannot cross the blood brain barrier. [$^{99m}$Tc]HMPAO concentration in the brain is stable and allows SPECT scanning to be performed several hours later, the image denotes brain uptake at the time of injection. This is important in patients that need sedation for imaging, as sedation will not induce changes in the image. It is also important in the study of seizure disorders, sleep abnormalities, etc. Another consideration is that rapidly after intravenous injection, blood circulating d,l,HMPAO converts into meso HMPAO, thus further entrance into the brain ceases completely (Nakamura et al., 1989).

HMPAO is available as a lyophilized kit, technetium pertechnetate is added and in presence of a stannous reductant a lipophilic compound is formed, the d,l diastereoisomer. This reaction occurs only with divalent, reduced tin, therefore any oxidant present in the pertechnetate will adversely affect the preparation. The [$^{99m}$Tc]HMPAO preparation is stable for 30 minutes when spontaneous conversion to the hydrophilic, meso, forms occurs. A variation of this compound, [$^{99m}$Tc] cyclobutil propilene amine oxime (CBPAO), that incorporates a butyl ring shows increased stability in vitro, for up to 6 hours while maintaining the instability in vivo (Colombo et al., 1989).

The usual patient dose is 20 mCi of [$^{99m}$Tc]HMPAO, injected IV, patients are injected in the supine position with low ambient light and noise. SPECT scanning starts 1 hour after injection in order

to clear soft tissue background activity from the soft tissues and muscles of the face and neck. At this time, there are no further environmental effects on the cerebral distribution of [$^{99m}$Tc]HMPAO. We compared [$^{99m}$Tc]HMPAO uptake to $^{133}$Xe rCBF in 20 patients using a brain dedicated system and the correlations over a wide range of rCBF are poor, ranging from r = 0.18 - 0.32. Therefore not only blood flow determines brain uptake of [$^{99m}$Tc]HMPAO but probably also BBB characteristics and individual conversion rate between lipophilic and hydrophilic forms.

Dosimetry for [$^{99m}$Tc]HMPAO has been estimated by Amersham Ltd., the manufacturer of the radiopharmaceutical using a few patients, the target organ being the lacrimal glands which receives 5.7 rads for a 20 mCi dose (Amersham, Ceretec™ package insert). We calculated lacrimal gland dosimetry on 138 patient studies. Lacrimal gland uptake was seen in 11% of patients and the dose to the gland was estimated to be 1.02 rad for a 20 mCi dose (Villanueva-Meyer et al., 1989). This reduction of radiation dose to the target organ justifies an increase of dose of [$^{99m}$Tc]HMPAO to 30 mCi. 30 % of the injected dose is found in the gastrointestinal tract immediately after injection. Over 48 hours 50 % of the dose is excreted through the gastrointestinal tract and 40 % trough the kidneys and urine (Amersham, Ceretec™ package insert).

**[$^{99m}$Tc]ECD**

99m-Technetium ethyl cysteinate dimer, [$^{99m}$Tc]ECD (Neurolite™, DuPont, Billerica, MA), is a lipophilic compound that crosses the blood brain barrier in the 1,1 form, it is retained in the brain by rapid deesterification: conversion from a diester to a monoester which is a polar metabolite (Wolovitch et al., 1988). Brain uptake is 6.4 $\pm$ 2.1 % of the injected dose 5 minutes after injection, falling slowly to 3.7 $\pm$ 0.3 % 4 hours later (Holman et al., 1989). Blood clearance is rapid, descending to 7.4 $\pm$ 3 % ID at 30 minutes, this results in higher brain to soft tissue ratios than have been reported with [$^{99m}$Tc]HMPAO. Clearance of the tracer is primarily by the urinary tract, and the target organ is the bladder, 0.11 rad/mCi assuming a 2 hr void (Holman et al., 1989).

In contrast to [$^{99m}$Tc]HMPAO, [$^{99m}$Tc]ECD it stable in vitro, up to 24 hours. The washout rate from the brain is 17 hours, compared to 71 hours for [99mTc]HMPAO. [$^{99m}$Tc]ECD produces better quality images because of higher gray-to-white-matter ratio. Also the soft tissue uptake in the face and neck is lower than [$^{99m}$Tc]HMPAO therefore the cerebellum, brainstem and temporal lobes can be defined more precisely.

**$^{123}$I-IMP**

The first $^{123}$I-amines as brain imaging agents were developed in 1980, they have a high brain extraction fraction and slow washout (Winchell et al., 1980). $^{123}$I-N-isopropyl-p-iodoamphetamine hydrochloride ($^{123}$I-IMP, $^{123}$I-iofetamine, Spectamine, Medi-Physics, Paramus, NJ) is available and approved by the Food and Drug Administration. However the metabolism of $^{123}$I-IMP is complex and not completely understood, multiple factors influence its brain accumulation: brain perfusion, lipophilicity, affinity to adrenergic receptors and blood brain barrier characteristics

(Baldwin and Wu, 1988; Holman et al., 1983). After IV injection there is rapid uptake by the lungs with release into the brain and other organs. The brain concentration rises slowly, it reaches maximal concentration at 30 minutes (6-7 % of the injected dose). Afterwards the $^{123}$I-IMP brain concentration remains in plateau for several hours, and starts falling slowly. In the brain $^{123}$I-IMP is either bound to nonspecific receptor sites or is converted to a nonlipophilic metabolite. Metabolism of $^{123}$I-IMP occurs readily in the brain, liver and lungs and implies dealkylation of the primary amine: loss of isopropyl moiety, to p-iodoamphetamine (PIA). Activity in the brain and lungs consist only of IMP and PIA 24 hours after administration. The rate-limiting step appears to be deamination to a ketone: producing the transitory form p-iodophenylacetone, which is rapidly degraded to p-iodobenzoic acid, then conjugated with glycine in the liver to give the end product which is p-iodohippuric acid, excreted through the kidneys in the urine (Baldwin and Wu, 1988). The distribution of $^{123}$I-IMP during the first 10 minutes correlates well with rCBF measurements, therefore perfusion imaging should be performed early. Later the brain distribution changes, probably reflecting metabolic activity and to a lesser extent perfusion. $^{123}$I-IMP studies require early and late imaging, 20 minutes and 4 hours after injection.

$^{123}$I may have a 2-5 % $^{124}$I (660 keV) contamination if produced by the low-energy reaction (23 meV) $^{124}$Te (p,2n) $^{123}$I. $^{124}$I contamination increases patients radiation dose and degrades image quality. $^{123}$I prepared using higher-energy photons (70 meV) and the reaction $^{127}$I (p,n5) $^{123}$Xe decays to $^{123}$I does not contain $^{124}$I being therefore the agent of choice. The injected dose of $^{123}$I varies from 3 - 6 mCi.

Disadvantages of $^{123}$I-IMP are related to low counting rate per slice. With an injected dose of 3 - 6 mCi the count rate is relatively low and affects resolution with a single cristal rotating gamma camera, therefore imaging with a dedicated brain scanner is more efficient. Dosimetry: the critical organ is the liver (0.17 rad/mCi) followed by the thyroid (0.1 rad/mCi), all this doses increase with $^{124}$I or $^{125}$I contamination (Baldwin and Wu, 1988).

The amount of iodoamphetamine injected is in the range of 0.45 to 0.9 mg, while only 1 - 2 mg may increase systolic blood pressure by 10 mmHg in hypertensive patients. 123IMP may be psychoactive in certain conditions, it should not be used while under treatment with mono amino oxidase (MAO) inhibitors and in patients hypersensitive to sympathicometic amines (McAfee, 1989).

## INSTRUMENTATION, ACQUISITION, PROCESSING AND DISPLAY PROTOCOLS

Developments in emission computed tomography equipment, for single photon (SPECT) and positron (PET) are in the areas of improved axial resolution, sensitivity, computational characteristics and clinical application. The ideal SPECT studies are performed with brain dedicated units with 8 mm resolution that approach PET resolution of 5 mm. SPECT is more commonly performed by a single headed rotating gamma camera, because of affordability and availability.

**Rotating gamma camera**

The following protocol is used for [$^{99m}$Tc]HMPAO acquisition and processing on a rotating gamma camera (Elscint 415 ECT, Haifa, Israel). A truncated detector equipped with a high resolution parallel hole collimator is used. The orbit is circular, over 360 degrees. 60 images are gathered every 6 degree steps, for 20 seconds each on a step and shoot modality. Total acquisition time is 20 minutes. The matrix size is 64 x 64. The counts per projection are in the range of 50,000 to 90,000 with a total number of counts for the whole series between 3 and 5 million. The 60 projections raw images are corrected for decay and smoothed. Uniformity correction is performed with a high count sensitivity map consisting of a 120 million count flood field image. The acquisition zoom used is 1.6 and the reconstruction zoom is 1.33. Reconstruction is performed by means of the backprojection algorithm using a Hanning filter. Attenuation correction using Chang's method is applied, and the attenuation correction coefficient is $0.125^{-1cm}$. A tridimentional matrix is obtained and transaxial, coronal and sagittal sets of images are extracted. Each set had 14 to 16 images depending on the volume of the brain. The images are 2 pixels thick = 1 cm. For the display we use a color scale that denotes abnormal diminished uptake by a drop below 66% of maximal encephalic activity.

**Multiprobe system for $^{133}$Xe rCBF measurement**

A multiprobe system is a non-imaging detector system that monitors $^{133}$Xe concentration in the brain. The unit may have up to 32 detectors, (Novo Diagnostic Systems, Cerebrograph 10a-32, Hadsund, Denmark) in a self-contained portable system that is easy to handle in bedside examinations. Collimated brain scintillation detectors are positioned over each hemisphere. Each NaI detector is 1 cm in diameter, they are integrated in a system that includes channel analyzers, data collection unit, a microprocessor and a matrix printer. The system is placed on a small cart that carries also an air sample pump and a trap for exhaled $^{133}$Xe. Flow values are available immediately after finishing the study in ml/100gr/min. Since the half-value for tissue absorption of $^{133}$Xe is 4 cm mainly the cortical flow is measured, the deep brain structures as the basal ganglia are not represented. The breathing system is either connected to a face mask for spontaneously breathing or to an endotracheal tube. The $^{133}$Xe concentration in the exhaled air is monitored either by an air detector and exhaled $^{133}$Xe is retained in a charcoal trap. After intravenous injection of 30 mCi of $^{133}$Xe, measurements of the $^{133}$Xe brain activity begins for 10 minutes. As there is overlap of grey and white matter flow seen by each detector they have to be separated by using a 2 exponential model, this can be achieved because there is a fourfold difference between grey and white matter flow (Kety, 1951; Obrist et al., 1967; Kohlmeyer, 1985). Fixed brain-blood partition coefficients are assigned to grey and white matter, the values are derived from measurements made in vitro from dissected brain (Veal and Mallet, 1965). Since the rCBF is calculated from the product of the flow rate constant and the partition coefficient it is desirable to measure both parameters in vivo since they may become altered with aging and disease (Kety, 1951). In vivo it is not possible to measure the partition

coefficient of $^{133}Xe$ and the fixed value used may cause errors in the final calculation, over or underestimation of values in the range below 20 %. Using a two compartmental analysis the washout rates for the fast (grey matter) and slow (white matter) components are calculated, and results expressed in ml/100gr/min. Under certain pathologic conditions overlap of both compartments may occur due to compartment size differences. A limitation of the multiprobe rCBF calculation concerns the incapability to detect areas of no flow or low flow, pathologic gray matter may behave as white matter and viceversa, which is known as slippage. In order to overcome the overlap of compartments non-compartmental indexes can also be calculates: the ISI, initial slope index (analyzes first 2 minutes of the clearance), estimates CBF as % of ml/100gr/min, and the $CBF_{15}$ which corresponds to the height/area integration for 15 minutes. There are some differences when comparing the rCBF values, the highest values were obtained with the two-compartmental method (so it is well suited for activation studies), ISI tends to underestimate high flow values (it has less sensitivity but better reproducibility, less variation especially under slippage conditions).

Normal values for rCBF reported for intraarterial $^{133}Xe$ injection are 60 $\pm$ 7.8 ml/100gr/min with an interregional coefficient of variance of 6.8 % (Kohlmeyer, 1985), this are similar to the values obtained by $^{133}Xe$ SPECT, 60 $\pm$ 11 (Lauritzen et al., 1981) and 58 $\pm$ 8 ml/100gr/min (Kreisig et al., 1987). In newborns, ages 3-31 days, with low birth weights but normal neurologic exam, the values for grey matter flow are 85 $\pm$ 4 ml/100gr/min (Younkin et al., 1988). With aging a slow decline in rCBF has been observed, estimated to range from 0.26 - 0.53 ml/100gr/min/year (Hagstadius and Risberg 1989; Lavy et al 1978; Meyer et al, 1978).

**Brain dedicated imaging device, $^{133}$Xenon-133 rCBF SPECT**

Imaging of a dynamic process with SPECT can be achieved with a system originally developed by Kuhl (Kuhl et al., 1976), a very sensitive device that gathers sequential tomographic images. We have imaging experience with a multiring system (Kanno et al, 1981) (Shimadzu, Set 031, Kyoto, Japan) and a four-faced digital rotating gamma camera (Stokely et al., 1980; Sveinsdottir et al., 1977) (Medimatic, Tomomatic 64 and 564, Hellerup, Denmark). The following description corresponds to the multiring imaging device. The system consists of 3 rings of 64 NaI detectors each, arrayed in a 42 cm diameter circle. A fan beam collimator with two symmetric halves is used, the collimator is designed for high sensitivity at the expense of resolution. The acquisition processes consist of movements of the collimator and the detector ring: the collimator rotates in 10 degree steps over 180 degrees, then the detector rings shift 0.7 degree and the 18 steps of the collimator movement are repeated, the detector ring moves 8 times, in 0.7 degree steps, to cover 5.6 degree which is one-64th of 360 degrees. 3 slices are gathered simultaneously, the first 2 cm above the orbito-meatal line with 2 cm interslice spacing. Sequential 1 minute images each are gathered for the 3 slices. The count rate at its maximum is 500000 cpm per slice. The acquisition has 3 phases: 1) A background acquisition is performed for 30 seconds. 2) Inhalation or equilibrium phase, 1 minute duration, $^{133}Xe$ is administered in a

closed system in a concentration of 30 mCi/liter in a total volume of 5 liters. The patient breaths through a mouthpiece with the nose clipped. 3) Washout phase of 4.5 minutes, were the patient breathes room air and exhales in the system that consists of 2 charcoal activated xenon traps. The tomograms are reconstructed using a filtered backprojection algorithm, attenuation correction is performed using the data from a reference uniform pool phantom. A $^{133}Xe$ time activity curve is obtained from the air the patient exhales. A count drop to 20% of maximal counts is defined in the exhaled air time activity curve and used as the initial point for the washout analysis in the brain. This is done on a pixel by pixel mode from the dynamic images. For the calculation of rCBF a single exponential model is used in contrast to the 2 exponential model of the multiprobe system (Kanno et al., 1979; Celsis et al., 1981). SPECT images separate gray from white matter and therefore no overlap occurs as seen with the multiprobe system. Display of 3 slices is achieved using a color scale that is coded to ml/100g/min. Thus rCBF can be read directly from the color image.

**Dedicated brain imaging device, [$^{99m}$Tc]HMPAO SPECT**

Imaging of [$^{99m}$Tc]HMPAO distribution in cerebral cortex can be optimally achieved with a brain dedicated imaging device and using high resolution collimation. Simultaneously 3 brain slices 16 mm thick and separated by 32 mm are gathered. Acquisitions are completed in 10 minutes and are followed by 2 bed shifts of 16 mm each that image the blind interslice space, thus acquiring in 30 minutes 9 transaxial brain slices, without blind areas in the total image. The injected dose of [$^{99m}$Tc]HMPAO and time of imaging is similar to that of the single crystal rotating cameras. The total number of counts of the series is 20 million, which is about 6 times more than what is obtained with a rotating gamma camera. Reconstruction is done with the back projection reconstruction algorithm using with Ramachandran and Butterworth filters. Attenuation and uniformity corrections are performed using a high count map previously acquired from a phantom. A resolution of 8 mm in the periphery and 9.6 mm at the level of the basal ganglia is achieved and compares favorably to the 15 mm resolution of the rotating gamma camera. 9 transaxial images are displayed with a color scale that defines the normal to abnormal cortical interphase at 66% of maximal counts of each slice. The analysis of basal ganglia requires different criteria for the definition of abnormalities, clear distinction of the internal capsule from the basal ganglia is achieved by using a cutoff at 80 % of maximal counts. Coronal and sagittal reconstructions complement the transaxial slices. The coronal slices are particularly useful to evaluate the temporal lobes while the sagittal slices evaluate the frontal lobes.

## CLINICAL APPLICATION

**Dementia**

The term dementia is a symptomatic classification to impairment in cognitive and memory functions without alteration in the level of consciousness. It is estimated that 5% of the individuals over the age of 65 and 20% of those over the age of 80 have dementia. This represents a major clinical problem as the elderly population is growing rapidly, it is estimated that by the

year 2000 20% of the population in the USA will be over 65 years of age. The most common cause of dementia is Alzheimer type dementia (DAT), a condition that is difficult to diagnose with noninvasive techniques. DAT is the cause of over 50-60% of the dementias while multiple infarct dementia (MID) occurs in 30%, only 10-15% of the dementias are treatable disorders (Cummings and Benson, 1983).

The limitation of clinical diagnosis of DAT are illustrated by many studies that report sensitivity that range between 0.69 to 0.87 with specificities between 0.73 to 0.78 (Chang Chui, 1989). The misdiagnosis rate of DAT varies between 0.07 to 0.57 being the most common problem depression (Siegel and Gershon, 1987). Severe depression clinically may present with similar features as dementia and originated the term pseudodementia. The role of PET and SPECT in depression has yet to be determined.

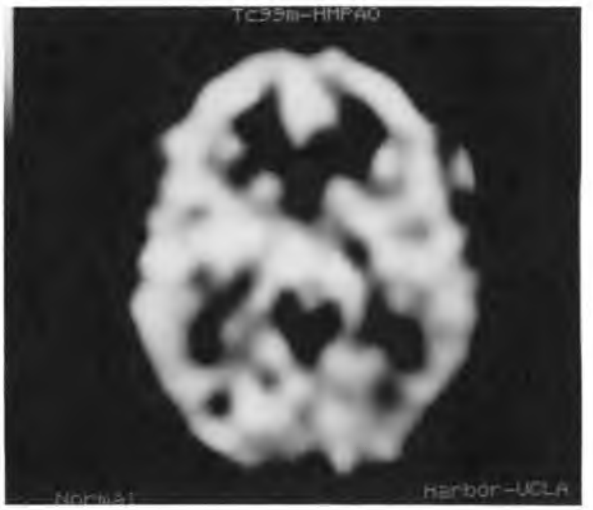



**Figure 1.** Selected transaxial slice at the level of the basal ganglia from a normal volunteer gathered with [$^{99m}$Tc]HMPAO SPECT.

At present for the study of the brain in dementias imaging with PET or SPECT are the best non-invasive techniques available. In DAT a particular pattern of bilateral temporoparietal abnormalities has been demonstrated with a variety of metabolic and flow tracers. The observation that metabolism and perfusion are coupled is frequent in brain imaging. The brain contains small amounts of energy generating substrates and the continuity of function, metabolism, is dependent on continuity of blood supply. With [$^{18}$F]fluorodeoxyglucose (FDG) bilateral parietal hypometabolism has been demonstrated (Friedland et al., 1983; Benson et al., 1983; Chase et al., 1984; Duara et al., 1986; Small et al., 1989), others have demonstrated a similar pattern with [$^{123}$I]IMP (Jagust et al., 1987; Johnson et al., 1987).

With SPECT imaging techniques we find also temporo-parietal hypoperfusion in DAT with [$^{99m}$Tc]HMPAO. Furthermore, $^{133}$Xe rCBF measurements show diffuse decrease of perfusion, in our series (45 patients with probable DAT by NINCDS and ADRDA criteria) the mean rCBF for females was 41±8 ml/100gr/min and for males 47±16 ml/100gr/min.

[$^{99m}$Tc]HMPAO SPECT shows bilateral perfusion defects in posterior parietal and temporal cortex, when compared to studies in normal volunteers (Figures 1 and 2). The abnormality was symmetric in 20/45 patients, in the asymmetric group left sided abnormalities were present in 15/45 and right sided abnormalities in 10/45 (Mena and Villanueva-Meyer, 1989). The asymmetry is related to different patterns of neuropsychological involvement, in predominant left sided parietal involvement, language was disproportionately impaired relative to visuospatial function and viceversa (Koss et al., 1985; Haxby, 1988). We found a significant number of areas of frontal hypoperfusion in DAT, 44 %, this defect has been associated with irritability and aggression (Garrett et al., 1988; Thompson et al., 1989). Histopathologic findings in DAT show severe abnormalities in temporal, parietal and frontal association areas, as well as in the limbic system, a relatively spared area is the sensory-motor cortex (Brun and Eglund, 1981). The highest concentration of neurofibrillatory tangles and senile plaques are found at necropsy in the mesial temporal cortex and posterior parietal area, however in the living patient these regions are usually avoided for biopsy because of proximity with mayor vascular structures (Cummings and Benson, 1983). The reason for the decreased rCBF in the temporo-parietal cortex in DAT is not known but may be related to decrease metabolic demand. Since rCBF is measured with $^{133}$Xe and reported in terms of ml per 100 gr of tissue per minute a generalized loss of brain substance should not reduce the flow, provided the metabolic demands of the remaining brain are normal.

The temporo-parietal hypoperfusion seems to be a sensitive indicator of DAT but its specificity is not clear. This pattern is distinctive as to allow separation of DAT from the healthy subjects and from multiple infarct dementia (MID) and is present in early and advanced DAT. But temporo-parietal hypoperfusion has been found also in some elderly individuals using $^{123}$IMP (Johnson et al., 1987), sleep apnea, posthypoxic dementia and MID using [$^{99m}$Tc]HMPAO (Miller et al., 1989). These findings suggest that the temporo-parietal cortex may be particularly sensitive to hypoxia and/or hypotension and that hypoperfusion of this area is not specific of DAT. In MID the abnormalities are multiple, do follow a vascular distribution and frequently are asymmetric. They are not concentrated in any particular area of the brain. They may also be located between the main vascular territories, in the 'watershed' areas. Abnormalities are demonstrated with CT and MR, hypodense areas and atrophy that match in location the SPECT findings. Although the SPECT lesions appear larger than the CT lesions in stroke. DAT patients tend to have normal CT and MR studies with some degree of diffuse cortical atrophy. Hachinski et al demonstrated that rCBF is lower in MID than in DAT, and that vascular reactivity to hypocapnia is retained in both MID and DAT (Hachinsky et al., 1975).

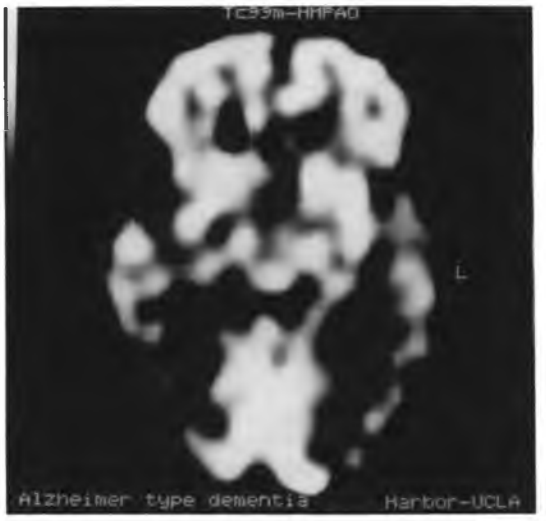



**Figure 2.** Transaxial slice from [$^{99m}$Tc]HMPAO SPECT in an Alzheimer type dementia patient. Note bilateral temporo-parietal hypoperfusion.

While the uptake of [$^{99m}$Tc]HMPAO is a function of perfusion, multiple attempts have been performed to quantify its uptake and relate it to absolute rCBF. To date this is not possible because of the complex uptake mechanism. In DAT it is possible to correlate the cortical to the basal ganglia uptake obtaining suboptimal sensitivities and specificities of 70 % and 75 % respectively (Leys et al., 1989). To quantify the cerebral uptake of [$^{99m}$Tc]HMPAO in sagittal frames we have developed a circumferential profile method based on the variation of uptake in the normal brain (Thompson et al., 1989). The sensitivity improves to 92 % while maintaining a specificity of 75 %. The method is semiautomatic requiring selection of the sagittal slices to be analyzed and definition of one point in the central area of the brain, in the ventricular system. The process continues automatically, a clockwise radial gradient algorithm detects then the inner and the outer boundaries of the cortex. A region of interest is created and the circumferential profile measures the activity pixel by pixel. The profile is displayed comparing it to a normal population. We use background substraction. A color coded functional image is created, and all pixel that have less than 2 SD of the maximal brain uptake are highlighted, counted and expressed as % of pixels in the region (Figure 3). In normal individuals less than 4 % of the pixels fall in this category. DAT patients show an average of high number pixels below 2 SD of maximal cortical uptake. We observe a correlation with neuropsychological testing, (patients with moderate MMS reduction, 28 to 15) with frontal lobe hypoperfusion (Mena et al; 1989). Notably among the correlations is the one with aggressive behavior. Others describe a correlation between the severity of disease with frontal hypoperfusion (Broich et al., 1989).

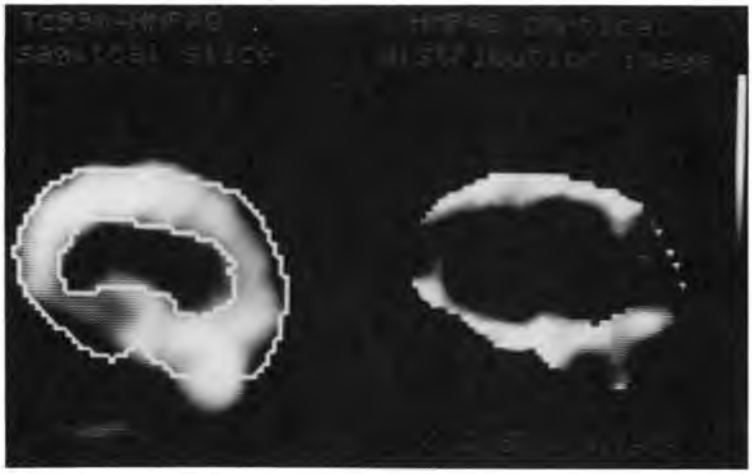


**Figure 3.** Sagittal image of [$^{99m}$Tc]HMPAO distribution in DAT patient (left). Pixels whose counts are below 2 SD of the maximum in the slice are abnormal and appear as black.

Semiquantitative evaluation of similar parameters in transaxial slices has been accomplished independendently by (Darcourt et al., 1989). A visual regional score of [$^{99m}$Tc]HMPAO uptake was developed and correlated with MMS and other psychometric scales, i.e. global deterioration scale (GDS) and Blessed A and B.

In 18 ADT patients scored by two independent observers their results correlated with a satisfactory $r = 0.95$, standard error = 1. A global score was defined as the sum of the individual regional scores and correlated with MMS $r = 0.84$, $p < 0.0001$; GDS $r = 0.73$, $p < 0.0004$, Blessed A $r = 0.58$, $p < 0.015$; Blessed B $r = 0.80$, $p < 0.0001$. Of interest is that the transaxial cuts demonstrated better correlation with MMS and impairment of perfusion of the temporal lobes $r = 0.79$, $p < 0.0001$ and the parietal region $r = 0.71$, $p < 0.0006$ while the posterior parietal regions yielded a correlation of $r = 0.62$, $p < 0.004$.

Thus, this information body of automatic quantitation analysis of [$^{99m}$Tc]HMPAO cortical uptake observed in sagittal cuts is confirmed for the transaxial cuts by semiquantitative analysis by two independent observers. The severity and extention of cortical hypoperfusion is a predictor of severity of disease as evaluated by psychometric testing. Therefore one can envision that actual localization of vascular hypoperfusion may lead to better classification of DAT, while these techniques are powerful and amenable to the evaluation of brain response to different stimulii.

Sequential $^{133}$Xe SPECT scans can be repeated every 15 minutes due to favorable dosimetry and to the short residence time of $^{133}$Xe

in the body, 90% of the administered dose is exhaled in the first 5 minutes. The sensitivity for detecting ischemic regions may be increased by provocative maneuvers as hypercapnia (Reich et al., 1989) or acetazolamide (Diamox), a carbonic anhydrase inhibitor used by injecting 1gr IV (Vostrup et al., 1984; Kreising et al., 1987; Hojer-Pedersen et al., 1987; Chollet et al., 1989; Rogg et al., 1989). Cerebral microvascular dilation is achieved and the reserve capacity can be assessed. These maneuvers cause vasodilation and potentially may rise intracranial pressure, therefore they should not be performed if the intracranial pressure is high. rCBF increases in normal individuals by performing a task or by pharmacologic intervention. Acetazolamide augmentation of rCBF in the normal individual ranges from 26 - 90 %, with advancing age the activation tends to be less than in younger individuals. The usefulness of acetazolamide is well documented in cerebrovascular disease but its role in dementia remains to be determined.

In conclusion, rCBF measurements with $^{133}$Xe and [$^{99m}$Tc]HMPAO in demented patients are useful in the differential diagnosis of dementia identifying DAT from other conditions: MID, and possibly depression, etc. The temporo-parietal hypoperfusion pattern is sensitive although non specific. Quantitative studies of frontal hypoperfusion correlate with psychometric testing, notably with aggressive behavior. With adequate quantitation of [$^{99m}$Tc]HMPAO uptake and possibly using activation studies the understanding in the diagnosis and classification of the dementias may be enhanced.

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